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# Summary Report

# The Causes and Control of Activated Sludge Bulking and Foaming

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## *Foreword*

The United States Environmental Protection Agency (EPA) has a responsibility to collect and transmit technical information that can be of use to members of the public involved in operations that contribute to increasing or maintaining the quality of the environment. In order to be most effective, this effort must be documented in a manner that facilitates the transfer of the technical information to the public for consideration and use. This report is an effort to provide a reference material on the causes and controls of sludge bulking and foaming in activated sludge treatment that can be readily understood, but also includes sufficient detail (in the appendices) to help plant operators control their systems.

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## *Acknowledgement*

Major portions of this document are taken in whole or in part from the recent EPA report, "Manual on the Causes and Control of Activated Sludge Bulking and Foaming," authored by David Jenkins, Michael G. Richard, and Glen T. Daigger under EPA Cooperative Agreement CR 811810.

# Chapter 1

## 1.0 Introduction

The reaction of wastewater with biologically active sludge to remove degradable pollutants from the wastewater is a widely applied treatment technique. The microscopic organisms in the sludge metabolize the degradable constituents of the waste, utilizing the products for growth and releasing less noxious by-products into the treated water.

The activated sludge treatment process consists of two basic unit operations: (1) aeration, and (2) clarification. These operations may be physically separated or, as in a Sequencing Batch Reactor (SBR) process, may take place in the same unit. In aeration, microorganisms are brought into contact with biodegradable wastes in the presence of a continuous oxygen supply. In clarification, the solids (consisting mostly of the active microorganisms and metabolic products) are allowed to separate from the liquid. Some of this activated sludge is then returned to the aeration tank, while the rest is removed from the system as waste activated sludge for further treatment and final disposal. The clarified liquid (effluent) flows from the clarifying tank to final processing (such as filtration or disinfection) before discharge.

The efficiency of this process depends upon the satisfactory functioning of both the biological oxidation and the solids separation processes. This report concentrates on two problems that inhibit satisfactory separation of sludge solids: (1) bulking, and (2) foaming. The report also discusses their causes and presents means by which they may be controlled.

## 1.1 Definition of Problems

Several common problems may arise in the separation of activated sludge from wastewater (see Table 1). Only two specific problems are considered in this report.

### *1.1.1 Sludge bulking.*

This is a condition in which the sludge becomes very light, increases in volume, and will not settle. The specific cause of sludge bulking considered here is the overabundance of filamentous microorganisms in the sludge that extend from the floc and interfere with the compaction and settling of the sludge. The effects of the bulking are a high Sludge Volume Index, low solids concentration in the return and waste sludge, and the hydraulic overloading of the solids handling systems. Under these conditions, floating sludge flows out of the tank with the effluent, producing a poor quality product.

### *1.1.2 Sludge foaming.*

This is a condition in which various kinds of foams appear on the surface of aeration and clarification tanks. One type of foam occurs during startup of activated sludge plants and usually disappears once the process is established. A more troublesome foam, heavy and brown, can accumulate on the surface of the aeration tank during normal operation, migrate to the clarification tank, and is then discharged with the effluent. It may become so thick that it overflows the clarification tank. As with bulking, the principal cause of this type of foaming is the overabundance of filamentous microorganisms. This report considers those foams caused by the presence of specific filamentous microorganisms in the floc, i.e., *Nocardia* ssp. and *Microthrix parvicella.*

The deleterious effects of sludge bulking and foaming occur mainly in the clarification tank, and result in degradation of effluent quality. The following is an overview of the component steps of the activated sludge process in which these two problems occur.

## 1.2 Overview of Typical Activated Sludge Process

### *1.2.1 Unit processes.*

After preliminary treatment (e.g., screening and grit removal) and primary treatment (settling), the influent wastewater in a typical activated sludge treatment plant flows into the aeration tank, along with activated sludge returned from the secondary clarification tank. In the aeration tank, the mixture is aerated, providing mixing to bring the wastewater and sludge into close contact and supplying oxygen to the microorganisms in the sludge. The bacteria digest the biodegradable components of the wastewater. The mixture of wastewater and sludge (mixed liquor) then flows to the clarification tank, where settling of the sludge occurs. The solids settle to the bottom of the tank, while the clear effluent is drawn off. Some of the settled

**Table 1. Causes and Effects of Activated Sludge Separation Problems**

| Problem | Cause | Effect |
|---|---|---|
| Dispersed growth | Microorganisms do not form flocs but are dispersed, forming only small clumps or single cells. | Turbid effluent. No zone settling of sludge. |
| Slime (jelly) Viscous bulking; (also possibly has been referred to as Non-filamentous bulking) | Microorganisms are present in large amounts of exocellular slime. In severe cases the slime imparts a jelly-like consistency to the activated sludge. | Reduced settling and compaction rates. Virtually no solids separation in severe cases resulting in overflow of sludge blanket from secondary clarifier. In less severe cases a viscous foam often is present. |
| Pin floc or Pinpoint floc | Small, compact, weak, roughly spherical flocs are formed, the larger of which settle rapidly. Smaller aggregates settle slowly. | Low sludge volume index (SVI) and a cloudy, turbid effluent. |
| Bulking | Filamentous organisms extend from flocs into the bulk solution and interfere with compaction and settling of activated sludge. | High SVI — very clear supernatant. Low RAS and WAS solids concentration. In severe cases overflow of sludge blanket occurs. Solids handling processes become hydraulically overloaded. |
| Blanket rising | Denitrification in secondary clarifier releases poorly soluble $N_2$ gas which attaches to activated sludge flocs and floats them to the secondary clarifier surface. | A scum of activated sludge forms on surface of secondary clarifier. |
| Foaming/Scum formation | Caused by (i) non-degradable surfactants and by (ii) by the presence of *Nocardia* spp. and sometimes by the presence of *Microthrix parvicella.* | Foams float large amounts of activated sludge solids to the surface of treatment units. *Nocardia* and *Microthrix* foams are persistent and difficult to break mechanically. Foams accumulate and can putrify. Solids can overflow into secondary effluent or overflow tank freeboard onto walkways. |

sludge is returned to the aeration tank, while the excess sludge is removed for further treatment and final disposal. Sometimes the return sludge is aerated in a separate tank before it is reintroduced to the main aeration tank, to reactivate the microorganisms by allowing them to complete consumption of waste remaining in the sludge.

### *1.2.2 Microbiological processes.*

The character of municipal wastewater is usually expressed in terms of two parameters: Five-day Biochemical Oxygen Demand ($BOD_5$) and Suspended Solids (SS). $BOD_5$ indicates the amount of oxygen that would be required for biological oxidation of the waste. It is determined by a standard test wherein a known amount of wastewater is mixed with a known amount of oxygen, and the mixture is incubated for 5 days at 20°C. During that time, bacteria in the wastewater use the oxygen to oxidize organic matter in the waste. At the end of that time, the oxygen remaining in the mixture is measured, and from the amount of oxygen consumed, the $BOD_5$ in milligrams per liter is calculated. SS is expressed in milligrams per liter, and is determined by filtering a sample of the wastewater and weighing the dried, solid-containing filter. (Standard Methods 208D).

The objective of the activated sludge treatment process is the reduction of both the BOD and SS of the wastewater. Bacteria in the sludge convert organic material in the waste to more stable material and inorganic byproducts ($CO_2$ , $H_2O$) and cell protoplasm in the presence of oxygen. In order for this process to proceed at optimum biological activity, the plant operator needs to determine the appropriate ratio to be maintained between the food supply (BOD) in the incoming wastewater and the mass of bacteria in the aeration tank.

Other biological conversions that can take place in the aeration tank are phosphorus removal and nitrogen removal. Both of these materials stimulate natural biological activity in the effluent-receiving waters, causing a decrease in dissolved oxygen and thus resulting in a decreased ability of receiving streams to support fish.

Nitrogen removal begins with the conversion of ammonia to nitrites and nitrates in the presence of oxygen (nitrification, conversion of Nitrogenous Oxygen Demand [NOD]). Then the liquid containing the nitrites and nitrates is placed under anaerobic conditions with an organic carbon source for the denitrifying bacteria (e.g., methanol or raw sewage) where the nitrites and nitrates can be converted to the $N_2$ gas that is lost from the liquid (denitrification). This two-step process is not accomplished at 100 percent efficiency—some ammonia remains in the effluent. Furthermore, in plants that disinfect effluent with chlorine, some ammonia in the effluent increases the amount of chlorine needed to bring the effluent within regulatory limits. Control of ammonia conversion is maintained by careful control of the level of oxygen in the aeration tank, and by aging of the sludge to ensure the presence of nitrifying bacteria.

Phosphorus is incorporated into the protoplasm of the new cells that are generated when the bacteria multiply as they oxidize the organic waste. Fifty to 90 percent of phosphorus in influent wastewater can be removed by the activated sludge process.

### *1.2.3 Physical plant.*

Wastewater enters the typical plant and receives preliminary treatment. This may include use of screens (when there are many large floating solids), comminutors (grinders to achieve size reduction), and grit chambers (when there are inorganic suspended solids). This is usually, but not always, followed by primary treatment in sedimentation tanks (for removal of settleable solids).

The wastewater then flows to the aeration tank. In some cases, the waste is mixed with return activated sludge at a point outside the aeration tank, and the mixture then enters the tank. In other systems, the waste and return sludge are mixed in the aeration tank. In the aeration tank, the mixture is aerated by either a diffused air system (in which compressed air enters the tank at the bottom of the tank, causing the tank's contents to circulate) or a mechanical aeration system (in which a blade or pump is used to agitate the surface or pump the mixture and toss it in the air, and to introduce oxygen and mix the sludge and wastewater). The size of the aeration tank is a function of the length of time that the waste will remain in the tank.

After a suitable contact period (e.g., 4 to 24 hours) the mixture of activated sludge and treated wastewater, called mixed liquor, flows into the secondary clarification tank, where the sludge settles out. This tank must be carefully monitored so that the settled sludge is removed at frequent enough intervals to prevent it from accumulating and flowing out with the effluent, and to prevent anaerobic conditions.

The settled sludge removed from the clarification tank is usually split into two streams. Some is returned to the aeration tank, and the rest is wasted and disposed, usually after thickening by sedimentation, centrifuging, or filtration. Sometimes the return activated sludge is reaerated to maintain aerobic conditions before its return to the aeration tank. The clear effluent flows from the clarification tank to be disinfected and discharged from the plant.

### *1.2.4 Batch and continuous flow processes.*

In a batch operation, material enters and leaves the tank only at the beginning and end of a full treatment cycle (including aeration and settling). All processes are performed in the same tank, but sequentially rather than simultaneously. The tank is filled, aerated for a set time, aeration is shut off, the solids are allowed to settle, and the clear supernatant is drawn off to allow room for the next "batch" of raw wastewater. A portion of the sludge is retained to seed the next cycle. The excess sludge is wasted.

Most large activated sludge plants use the continuous flow process. In this process, the wastewater flows continuously from one tank to another through the system with a discrete process occurring in each unit. Each process is therefore occurring continuously and simultaneously with the other major treatment processes. In such a system, a continuous flow of food (wastewater) and return activated sludge enters the aeration tank, and a continuous flow of effluent and settled activated sludge leaves the clarification tank.

# Chapter 2

## 2.0 Activated Sludge Floc

The activated sludge process produces a mass of active microorganisms, which agglomerates and flocculates in the system's aeration tank and secondary clarification tank, and which is capable of oxidizing organic matter in wastewater. Many of the operating problems that occur in activated sludge systems can be attributed to various characteristics of the activated sludge floc. This section presents a description of the composition and contents of the floc.

## 2.1 The Floc—Contents and Components

Activated sludge floc is normally composed of a widely varied assortment of microorganisms and particle sizes. Particles range from single bacteria, measuring 0.5 to 5 m, to large flocs measuring 1 mm (1,000 $\mu$m) or more.

The primary active population of the floc is heterotrophic bacteria (those that feed on organic matter), including *Pseudomonas, Achromobacter, Flavobacterium, Alcaligenes, Arthrobacter, Citromonas,* and *Zooglea.* Also present in smaller quantities are fungi, protozoa, and metazoa. Flocs also contain organic and inorganic particles from the influent wastewater, "extracellular polymers" that seem to promote bioflocculation, and volatile matter.

Researchers have suggested that there are two levels of structure in floc. The "microstructure," which is the basis for floc formation, consists of bacteria that are able to "stick to" one another by microbial aggregation and bioflocculation. The "macrostructure," which consists of filamentous microorganisms, forms a large network to which the floc-forming bacteria of the microstructure can cling.

## 2.2 Filamentous Microorganisms

If a particular sludge contains only microstructure bacteria, and no macrostructure (filamentous) microorganisms, flocs are small (averaging approximately 75 $\mu$m), round, and easily broken up in the aeration tank. This results in an operational problem known as "pin floc." Such floc will settle rapidly, but will leave large amounts of smaller particles still suspended in the effluent.

Thus, filamentous (macrostructure) microorganisms are extremely important to a good floc and efficient production of a clear effluent (see Figure 1).

### *2.2.1 Effect of filamentous microorganism count on floc.*

When filamentous microorganisms are present in activated sludge, they form a network to which the smaller floc-forming bacteria can cling. This promotes the formation of larger flocs, which do not readily break apart in the aeration tank, and which settle more efficiently, mechanically removing smaller particles as they settle. These large flocs are irregular in shape, instead of small and spherical.

Activated sludge floc can have a good balance of filamentous and floc-forming bacteria, giving good settling characteristics and a clear effluent; have too few filamentous microorganisms, resulting in pin floc and a poor-quality effluent; or have too many filamentous microorganisms, resulting in bulking (see Figure 2).

### *2.2.2 Examination of filamentous microorganisms in floc.*

In order to effectively manage operational problems caused by filamentous microorganisms, it is important to know not only how many are present, but also what types (see Section 3). Microscopic examination of floc will yield data on microorganism number and type, and on physical floc characteristics that influence settling. Appendices 1 and 2 present specific information about methods for performing such examination, including procedures for sampling, staining, counting, and identifying organisms.

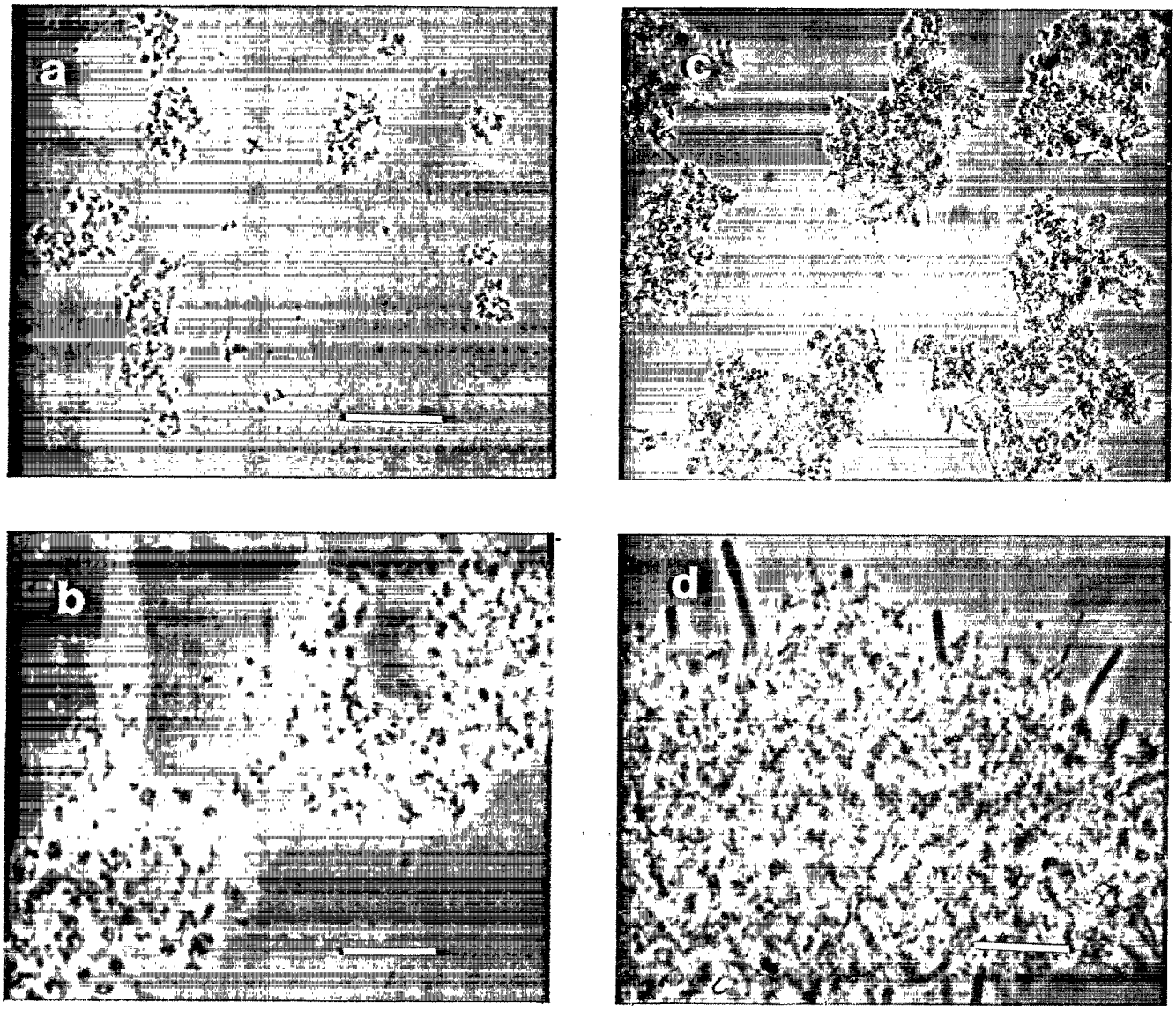
## 2.3 Definitions of Terms Used for Quantitative Description of Floc

Several quantitative parameters exist for the description of activated sludge floc. These parameters are defined below.

### *2.3.1 Mixed Liquor Suspended Solids (MLSS).*

The contents of the aeration tank is referred to as mixed liquor. It contains suspended microorganisms and other sludge components. MLSS is an expression, in mg/l, of the amount of microorganisms suspended

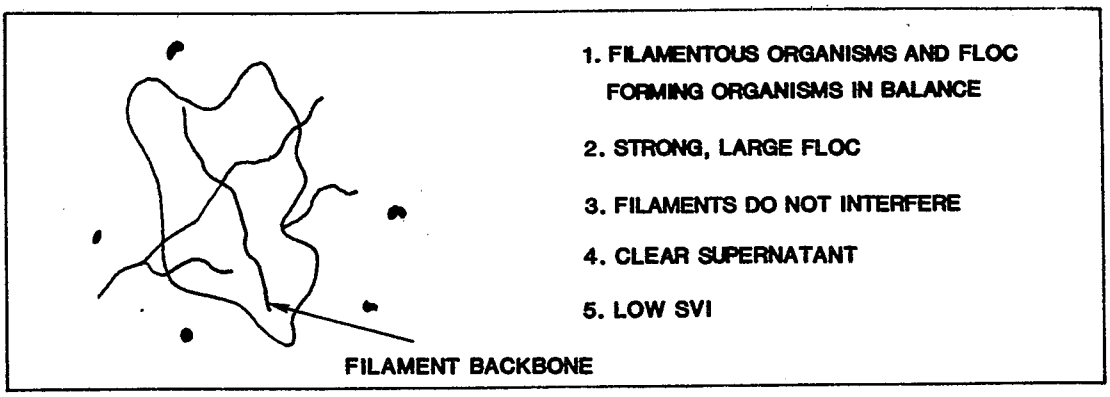
Figure 1. Microscopic appearance of activated sludge flocs: *a.* small, weak flocs (pin-floc) (100 × phase contrast); *b.* small, weak flocs (100 × phase contrast); *c.* flocs containing microorganisms (100 × phase contrast); *d.* floc containing filamentous microorganisms "network" or "backbone" (1000 × phase contrast) (*a* and *c* bar = 100 $\mu$m; *b* and *d* bar = 10 $\mu$m).



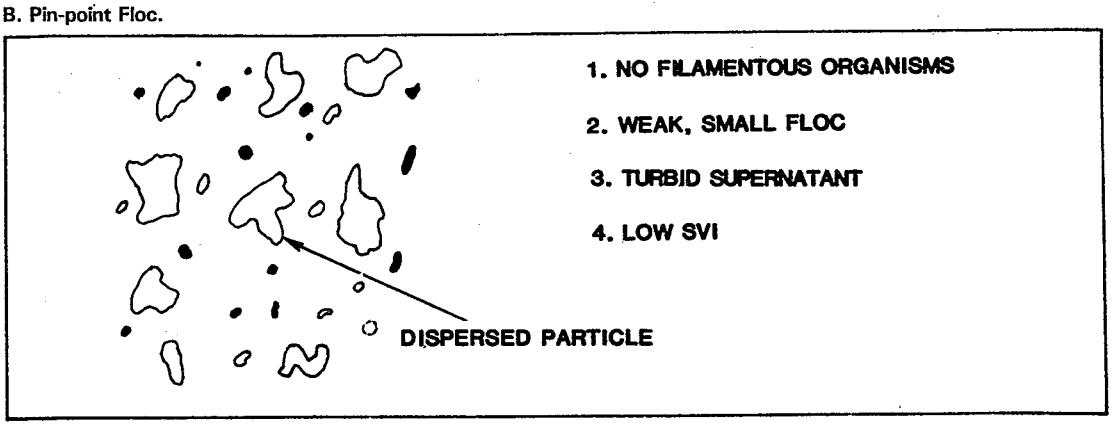

Reference: Jenkins, *et al.*, 1984.

Figure 2. Effect of filamentous microorganisms on activated sludge floc structure.

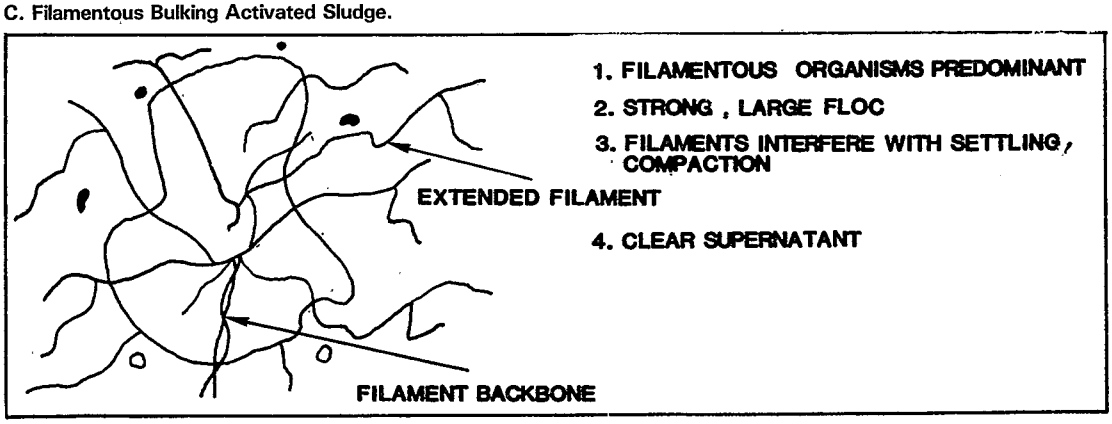
A. Ideal, Non-bulking Activated Sludge Floc.




B. Pin-point Floc.




C. Filamentous Bulking Activated Sludge.




Reference: Jenkins, *et al.*, 1984.

in the mixed liquor, in mg/l, and is a measure of the amount of microorganisms available to degrade the waste, or of the strength and density of the mixture as it flows from the aeration tank to the clarification tank. This parameter is determined by filtration of a known volume of mixed liquor, and drying and weighing of the filter and solids. (Standard Method 209C).

*2.3.2 Sludge Volume Index (SVI).*
This value measures the settling characteristics of the floc (sludge), and aids in determining if any adjustments should be made in operations.

SVI is the volume (in ml) of one gram of sludge after 30 minutes of settling. It is determined by taking one liter of mixed liquor from a point near the outlet of the aeration tank, allowing the sample to settle for 30 minutes in a graduated cylinder, and performing the following computation:

$$SVI = \frac{\text{volume (sludge) after 30 min. (ml/l)} \times 1000}{\text{MLSS (mg/l)}} = \frac{\text{ml}}{\text{g}}$$

SVI is a measure of the settling characteristics of the sludge—a sludge with a low SVI will settle and compact well. However, an SVI that is too low is indicative of pin floc. A high SVI is indicative of bulking.

*2.3.3 Sludge Age.*
This parameter measures the average number of days that solids remain in the system.

Sludge Age is computed using known parameters:

$$\frac{\text{Vol. aer. tank (gal.)} \times \text{MLSS (mg/l)}}{\text{Recycle flow rate (gal./day)} \times \text{recycle MLSS (mg/l)}} = \text{days}$$

A similar parameter, Sludge Retention Time, is computed by determining the mass of microorganisms under aeration or reaeration in the system, and dividing that value by the mass of microorganisms released in the waste activated sludge and lost in the effluent.

Sludge Age is important because it aids the operator in maintaining a viable population of microorganisms. The common range for Sludge Age in a conventional activated sludge plant is 3 to 15 days. Optimum Sludge Age can vary seasonally, as biological activity increases in summer and decreases in winter. Thus Sludge Age should generally be adjusted at least twice a year, raised in the winter and lowered in the summer.

A Sludge Age that is too low can cause floc to be light and fluffy, settling slowly or escaping with the effluent (a bulking condition). A high Sludge Age (too many solids in the system) can result in pin floc and a turbid effluent.

*2.3.4 Dissolved Oxygen (DO).*
This parameter is a measure of the amount of dissolved oxygen present in the process tanks. A minimum of 0.5 mg/l of DO should be maintained in the aeration tank. DO must be carefully monitored and controlled to ensure efficient operation. This monitoring may be accomplished with a portable oxygen meter or by lab analysis of samples (the former is preferred because there is no delay in obtaining results). Ideally monitoring takes place continuously at several points in the aeration tank.

*2.3.5 Food/Microorganism Ratio (F/M).*
This parameter indicates the ratio of food entering the system to the mass of microorganisms being aerated. It is computed as follows:

$$F/M = \frac{\text{pounds per day of BOD added to aeration tank}}{\text{MLSS in aeration tank (lbs)}}$$

The normal range for F/M for a conventional plant is 0.15 to 0.5.

## 2.4 Effect of Number of Filamentous Microorganisms on Quantitative Floc Parameters

SVI is the parameter most closely related to the number of filamentous microorganisms in the floc. Pin floc (floc with very few filamentous microorganisms) will have an SVI below 70 ml/g, while bulking sludge (with high numbers of filamentous microorganisms) will have an SVI above 150 ml/g.

Similarly, filamentous microorganisms affect the amount of suspended solids in the effluent. Sludge having few such microorganisms and thus resulting in pin floc will yield a turbid effluent, containing many suspended solids. A bulking sludge with too many filamentous microorganisms will result in a very clear effluent, but as the effluent flows from the clarification tank it will carry the large, light flocs, increasing the SS in the effluent.

## *Chapter 3*

## 3.0 Activated Sludge Bulking

Sludge bulking is one of the most common operational problems experienced by activated sludge treatment plants.

## 3.1 Symptoms of Sludge Bulking

Sludge bulking has visible symptoms as well as effects on the parameters used to assess the functions of the activated sludge system.

### *3.1.1 Qualitative manifestations.*

Sludge bulking occurs when sludge in the clarification tank gains in volume and becomes light and fluffy, flowing out over the weirs with the effluent. The effluent itself is otherwise very clear.

### *3.1.2 Quantitative manifestations.*

The most conspicuous quantitative symptom of sludge bulking is an elevated Sludge Volume Index (SVI). Bulking sludge generally has an SVI of over 150 ml/g. The density of bulking sludge is much lower than that of "ideal" sludge, and therefore fewer microorganisms are returned to the aeration tank in the return activated sludge (RAS). This interferes drastically with treatment efficiency, because fewer microorganisms are present in the aeration tank to digest organic matter.

## 3.2 Role of Filamentous Microorganisms in Sludge Bulking

As discussed in Section 2.2, filamentous microorganisms play an important role in the flocculation of activated sludge, forming a "macrostructure" to which smaller bacteria can cling (see Figure 3). Without filamentous microorganisms, pin floc can develop.

However, if there are too many filamentous microorganisms, floc will be diffuse and will settle poorly, resulting in bulking.

### *3.2.1 Relationship of filamentous microorganism count to bulking.*

The several methods of expressing counts of filamentous microorganisms are discussed in Appendix 1. In general, a filamentous microorganism count that exceeds a known standard, generated through experimentation and observation, will result in a high SVI and a bulking condition in the sludge (see Figure 4). Different filamentous microorganisms (and mixes of such organisms as occur in activated sludge) do differ in the number of such organisms necessary to impart a bulking condition to the sludge.

### *3.2.2 Relationship of specific filamentous microorganisms to bulking.*

Researchers have developed correlations between bulking and various specific filamentous microorganisms. Further, they have shown that different microorganisms that can cause bulking flourish when different operating problems are present. These correlations are summarized in Table 2. The research that resulted in this table gathered data on plant operating conditions only incidentally; it was focused on determining the presence of various microorganisms in bulking sludge. However, the table can be used to determine probable operational causes of bulking, if the dominant microorganisms in the bulking sludge are identified. (See Appendix 2 for a guide to microscopic identification of filamentous microorganisms.)

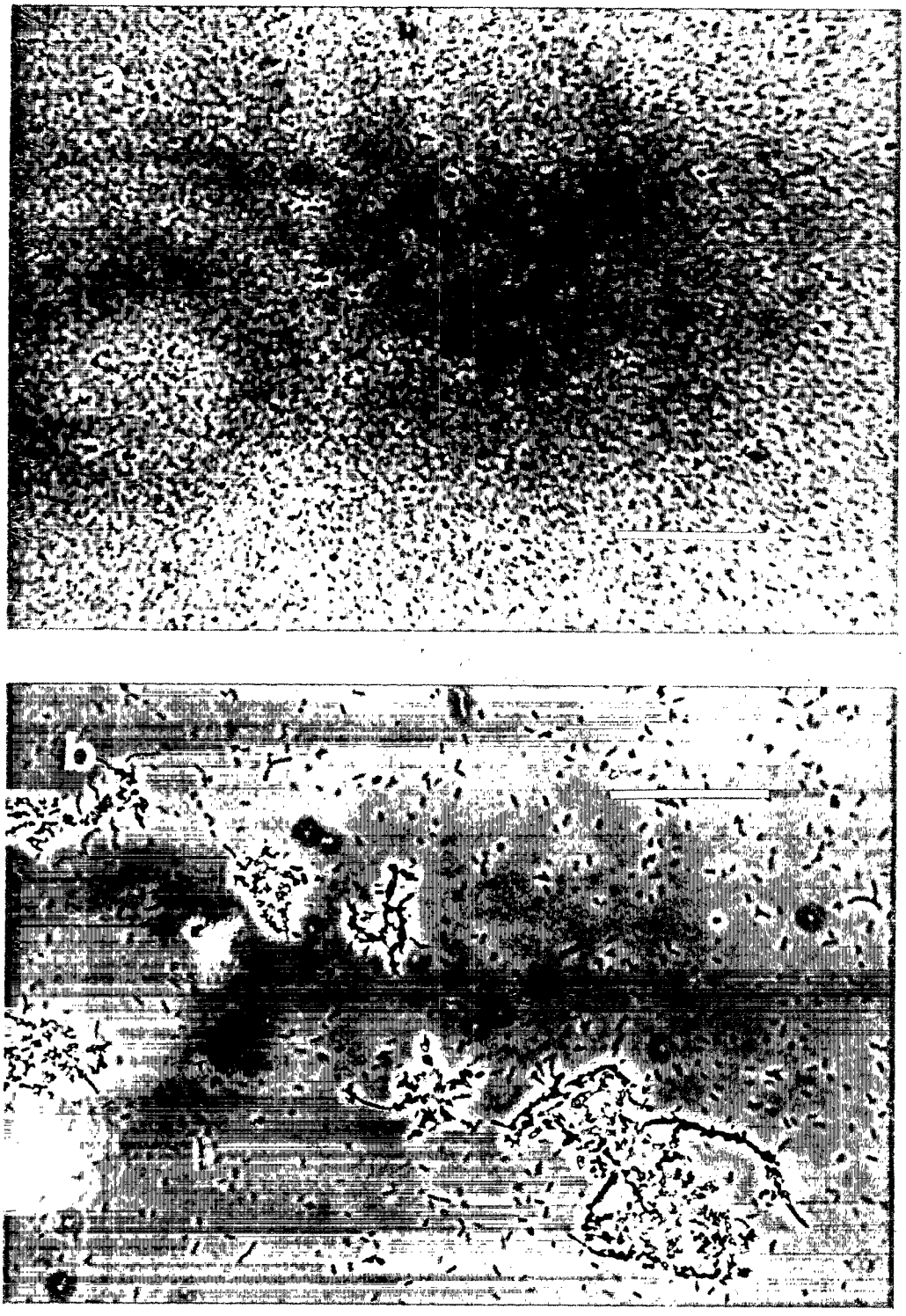
## 3.3 Factors Affecting the Presence of Filamentous Microorganisms

As Table 2 shows, changes in plant operating conditions, and resultant changes in the quantitative parameters defined in Section 2.3, can promote the growth of different filamentous microorganisms.

A low pH in the aeration tank can promote the growth of fungi. Based on laboratory experiments, the best activated sludge performance can be obtained at pH values from 7.0 to 7.5, although removals of oxygen consumed, suspended solids, and bacteria are good at pH values ranging from 6.0 to 9.0. The presence of fungi indicates that the wastewater most likely contains strong acid discharges and an aeration tank pH value below 6.0.

Low DO in the aeration tank can promote the growth of *S. natans* and type 1701. Low DO concentration in low F/M systems can promote the growth of *H. hydrossis*. Experiments have shown that the DO concentration required to prevent the growth of low DO filamentous

Figure 3. Effect of filamentous microorganisms on floc structure: *a.* aggregates observed for a pure culture of an activated sludge floc former; *b.* aggregates observed for a dual culture of a single floc former and a single filamentous microorganism (100× phase contrast; bar = 100 μm) (Lau *et al.*, 1984).




Reference: Jenkins *et al.*

Figure 4. Correlation of filament count and SVI for secondary (first stage) and tertiary (second stage) activated sludge systems at the San Jose/Santa Clara Water Pollution Control Plant, CA (Beebe *et al.*, 1982).

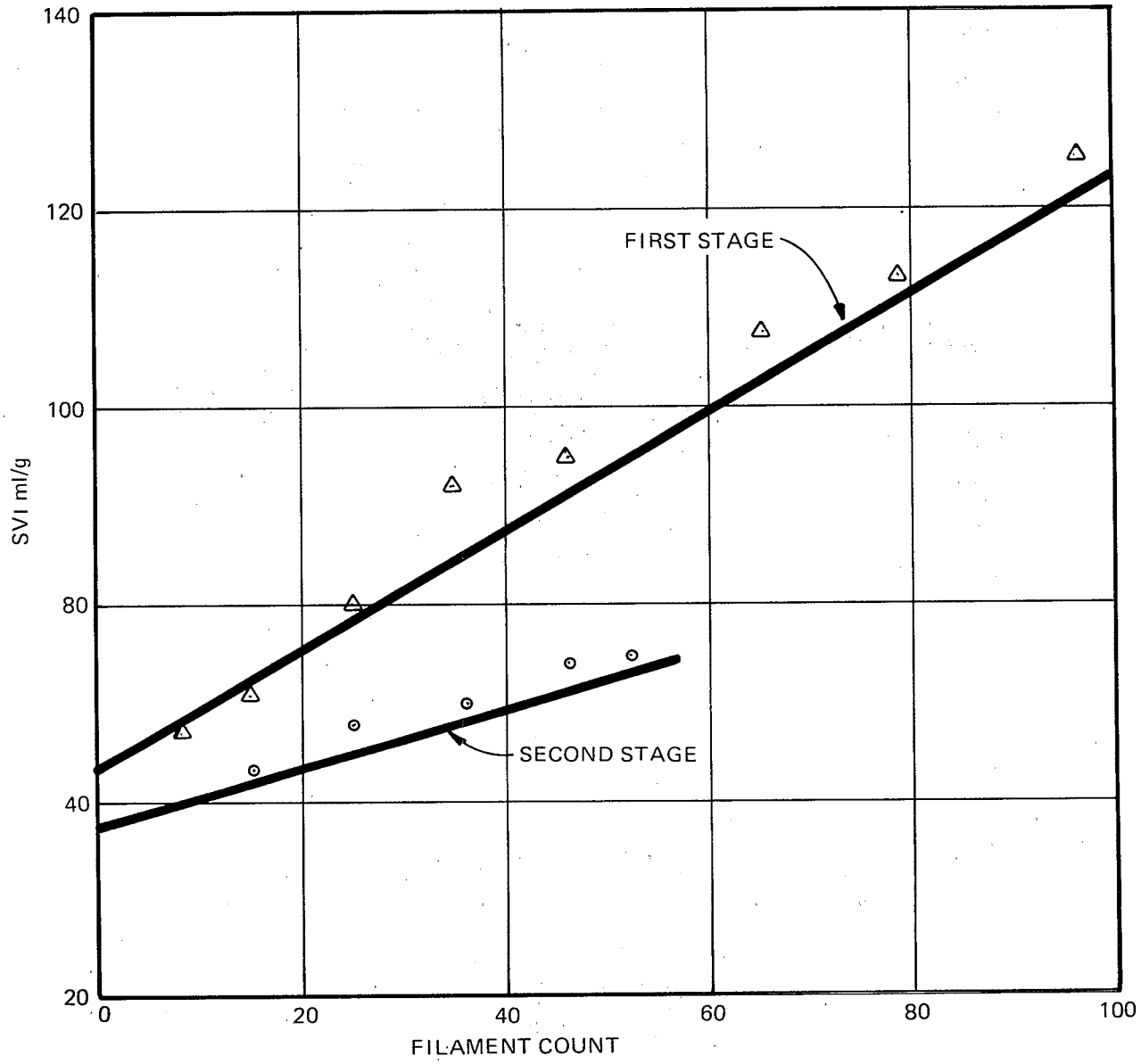


Table 2. Dominant Filament Types as Indicators of Conditions Causing Activated Sludge Bulking

| Suggested Causative Conditions | Indicative Filament Types |
|---|---|
| Low DO | type 1701, *S. natans, H. hydrossis* |
| Low F/M | *M. parvicella, H. hydrossis, Nocardia* sp types 021N, 0041, 0675, 0092, 0581, 0961, 0803 |
| Septic Wastewater/Sulfide | *Thiothrix* sp., *Beggiatoa* and type 021N |
| Nutrient Deficiency | *Thiothrix* sp., *S. natans*, type 021N, and possibly *H. hydrossis* and types 0041 and 0675 |
| Low pH | fungi |

Richard *et al.* 1982*a;* Strom and Jenkins 1984.

microorganisms was a function of the F/M load. The greater the F/M load, the greater the DO concentration required to prevent the low DO bulking.

Experiments have also shown that low DO bulking can be caused and cured by manipulation of F/M load and DO concentrations. However, in dealing with low DO bulking problems, a basic economic question must be addressed. To cure the problem, one must lower the F/M or increase the aeration tank DO concentration. Each of these actions may have undesirable consequences. Lowering the F/M may cause the onset of nitrification and increase MLSS levels to values that exceed the solids capacity of the secondary clarifiers. Increasing the aeration tank DO concentration will require greater power input and may also cause the onset of nitrification. Because of these factors, it is necessary to consider the alternative of operating at a low aeration tank DO concentration and killing off the filamentous microorganisms that develop using RAS chlorination. RAS chlorination is discussed in Section 3.4.4.1.

Low F/M load may result in *M. parvicella* and types 0041, 0675, 0581, 0961, 0803, and 0092. The specific causes for the growth of filamentous microorganisms that appear in continuously fed, completely-mixed, low F/M load activated sludge systems are poorly understood. These systems produce poorer settling activated sludge than systems that are fed intermittently or have aeration tanks where there is a relatively high local concentration of wastewater at the point where the RAS and influent enter the tank. Emperical measures producing a carbonaceous substrate concentration gradient in the aeration tank, or a high substrate concentration at the point where RAS and influent enter the aeration tank have consistently controlled low F/M induced bulking in laboratory studies, but less effectively in practice. The correlation of cause and effect between low F/M load and the presence of filamentous microorganisms is not completely understood and is being studied further.

Treatment of septic waste can lead to growth of *Thiothrix* spp. and type 021N. This may result from the ability of these microorganisms to grow on inorganic reduced sulfur compounds and organic acids, which are produced by fermentation in septic sewage. Under such conditions these microorganisms produce intracellular sulfur granules that are convenient for identification purposes.

Treatment of nutrient-deficient wastes (low in nitrogen or phosphorus) can lead to growth of *Thiothrix* spp. and type 021N, and possibly types 0041 and 0675. Under these conditions, *Thiothrix* spp. and type 021N do not contain intracellular sulfur granules, but may contain other intracellular granules such as polyhydroxybutyrate (PHB). Also under nutrient-deficient conditions, types 0041 and 0675 may show a Neisser-positive extracellular slime covering, although they normally stain Neisser negative (see Appendix 1 for stain procedures).

When microscopic examination suggests nutrient deficiency, the BOD, COD, or TOC to nitrogen and phosphorus ratio of the influent should be checked. Generally, sufficient nutrients are present when the influent to be treated by activated sludge contains $BOD_5$/N/P in the ratio of 100/5/1. If necessary, the required nutrients should be added. Long sludge age systems can treat wastes with less nitrogen and phosphorus than indicated by this ratio because of the recycling of nutrients by the endogenous decay of activated sludge in these systems.

While the $BOD_5$/N/P ratio is a useful guideline for detecting nutrient deficient wastes, the following factors also should be taken into account:

- The availability of nitrogen and phosphorus in the influent should be high enough for use during the metabolism of the carbon source.
- The nutrient supply should be paced so that nutrients never run out in any part of the aeration tank as a result of shock from influent carbon loads.
- Because each wastewater/activated sludge combination has its own unique nutrient demand, an influent $BOD_5$/N/P ratio should be checked by measurements of effluent concentrations of dissolved (0.45 mμ filtered) orthophosphate, ammonia, and nitrate.
- Ammonia and nitrate are available as nitrogen sources for activated sludge growth. Ammonia added in nitrifying systems for nutrient supplemation will be converted to nitrate and still remain available as a nitrogen source.

A Sludge Age that is too low can result in "straggler floc," light buoyant particles that do not settle in the clarification tank and flow over the weirs in an otherwise clear effluent.

## 3.4 Control of Activated Sludge Bulking

Maintenance of optimum levels of filamentous microorganisms in activated sludge is necessary to ensure efficient settling. Although proper treatment plant design is the best means of preventing the excessive growth of filamentous microorganisms that cause bulking, some design innovations are very recent, and many existing activated sludge plants do not have them. In such plants, bulking sludge must be corrected by adjustments in operations or by addition of chemicals.

### *3.4.1 General approach to controlling filamentous microorganisms.*

A good general approach to controlling bulking problems is as follows: First, identify the filamentous microorganisms causing the bulking (for identification procedures, see Appendices 1 and 2). Second, using the information in Table 2 and familiarity with conditions at the plant in question, determine the probable cause of bulking. Third, determine whether the underlying problem can be immediately rectified by operational changes (such as prechlorination of septic waste, addition of missing nutrients, or other adjustment of parameters). Fourth, determine whether the plant requires major design or operational changes that might take a long time to implement (such as changes in aeration capacity or unit configuration). If the plant does require such changes, sludge settling will not immediately become normal when those changes are made, but will improve only slowly because sludge remains in the system for an extended period of time (it could take as long as three times the Sludge Retention Time for settling to become normal). Because of the delays associated with major design and operational changes, methods are needed for rapid correction of the problem while such changes take effect. These methods include process manipulation, addition of chemicals to enhance settling, and addition of toxicants to selectively kill filamentous microorganisms.

### *3.4.2 Process manipulation to control bulking.*

In many cases, the adverse effects of bulking sludge can be minimized by proper management of the activated sludge system, particularly if it is underloaded or if certain process options were built into the original design. To use these process management tools to deal with bulking sludge, it is necessary to:

- Study in detail the technical operating principles of the activated sludge clarification tank, particularly as they relate to the thickening function;
- Examine specific techniques for determining the solids handling capacity of the clarification tank and how this is influenced by sludge settling characteristics; and
- Review techniques of clarification and aeration tank operation, in order to maximize clarification tank capacity in systems with bulking sludge.

#### *3.4.2.1 Design Calculations for Process Modifications*

Bulk sludge problems can often be corrected by changes in design parameters. This section reviews system operations from a quantitative perspective, and demonstrates calculations for changes in design.

*Clarifier Operation Principles.* Activated sludge clarification tanks have two basic functions—clarification and thickening. Clarification is the removal of activated sludge flocs to produce a clear overflow that either meets discharge standards or

does not overload downstream processes. Thickening of the settled activated sludge solids (i.e., RAS) is required so that they can be returned to the aeration basin. If the activated sludge solids are not thickened and removed from the clarification tank at a rate faster than they are added, they will accumulate until the clarification tank is full and the excess solids will be discharged into the effluent. Since bulking affects the ability of the activated sludge solids to thicken, the thickening function and thickening capacity of the clarification tank are affected by sludge bulking.

*Process Schematic and Definitions.* Figure 5 is a process schematic of the activated sludge system. The aeration tank has a volume V, and the clarification tank has a surface area A. Wastewater enters the aeration tank at flowrate Q, together with RAS, at flowrate $Q_r$. The aeration tank suspended solids concentration (MLSS) is X, and the RAS, SS is $X_u$. Activated sludge soilds are applied to the clarification tank at a flowrate of $Q + Q_r$.

It is assumed here that sludge is wasted from the RAS at a flowrate $Q_w$, at the same SS concentration as the RAS, $X_u$. Effluent is discharged from the clarification tank at a flowrate of $Q - Q_w$, and it contains an SS concentration of $X_e$.

*Process Operating Relationships.* In developing relationships to describe the clarification tank thickening function, certain simplifying assumptions can be made. First, the WAS flowrate ($Q_w$) is usually small compared to the influent flowrate (Q), and its effect on effluent flow can be neglected. Second, the quantities of activated sludge solids discharged in WAS and in the effluent are small compared to the quantity of sludge solids applied to and withdrawn from the clarification tank.

Using these assumptions and assuming that solids are not accumulating in the clarification tank, a simplified solids mass balance over the clarification tank is:

$$(Q + Q_r)X = Q_r X_u \qquad (1)$$

Equation 1 states that, at steady-state, the rate at which solids are applied to the clarification tank must be equal to the rate at which they are removed. Equation 1 can be used to develop the following relationships that are useful for assessing clarification tank operation.

*1. Degree of Thickening that can be Achieved by a Clarification Tank*

$$\frac{X_u}{X} = \frac{(Q + Q_r)}{Q_r} \qquad (2)$$

This relationship illustrates that the degree of thickening is a function of the influent and RAS flowrates.

*2. Calculation of Required RAS Flowrate*

$$Q_r = \frac{Qx}{(X_u - X)} \qquad (3)$$

This relationship can be used to calculate the RAS flowrate required for a specified influent flowrate, the current aeration tank MLSS concentration, and an RAS SS concentration that it is believed (perhaps based on past history) can be achieved in the clarification tank.

*3. Clarifier Capacity Calculation*

$$Q = \frac{Q_r(X_u - X)}{X} \qquad (4)$$

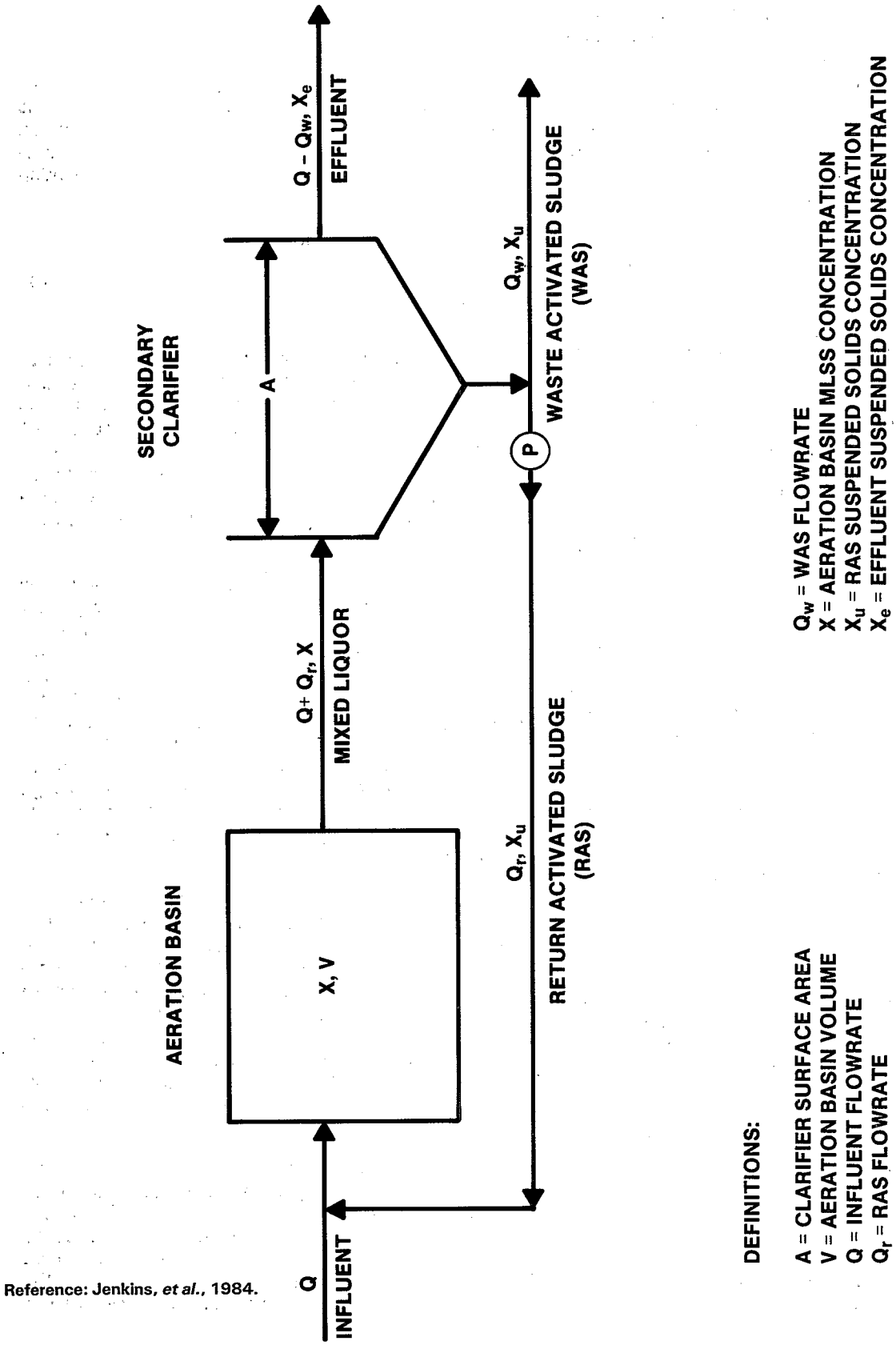
This relationship can be used to calculate the capacity of a clarification tank for a specified RAS flowrate and an aeration tank MLSS concentration, and for an achieveable RAS/SS concentration. Equations 1–4 assume that sufficient clarification tank area is available to thicken the RAS to a concentration of $X_u$. Techniques for determining the clarification tank area required to achieve this are discussed in the following section.

*Sludge Thickening Theory.* Thickening of activated sludge solids in a clarification tank occurs by gravity settling. As the solids settle, their concentration increases from the MLSS concentration (X) to the RAS concentration ($X_u$). Because of their flocculent nature and concentration, activated sludge particles do not settle individually, but as an entire mass. The solids settle at a constant rate until they begin to "pile up" at the bottom of the container in which they are settling. The initial settling velocity of the activated sludge solids ($V_i$) decreases as the initial SS concentration increases. This type of behavior is called zone settling, or type III settling.

Zone settling is familiar to anyone who has run an SVI test. The entire mass of activated sludge settles together, producing a well-defined interface between the top of the settling sludge and the clear supernatant. When the height of the interface is plotted against time, the line initially is straight but later begins to level off (Figure 6). The slope of the initial straight line is the initial settling velocity, $V_i$. Sludges with higher initial SS concentrations settle slower than those with lower initial SS concentrations (Figure 6).

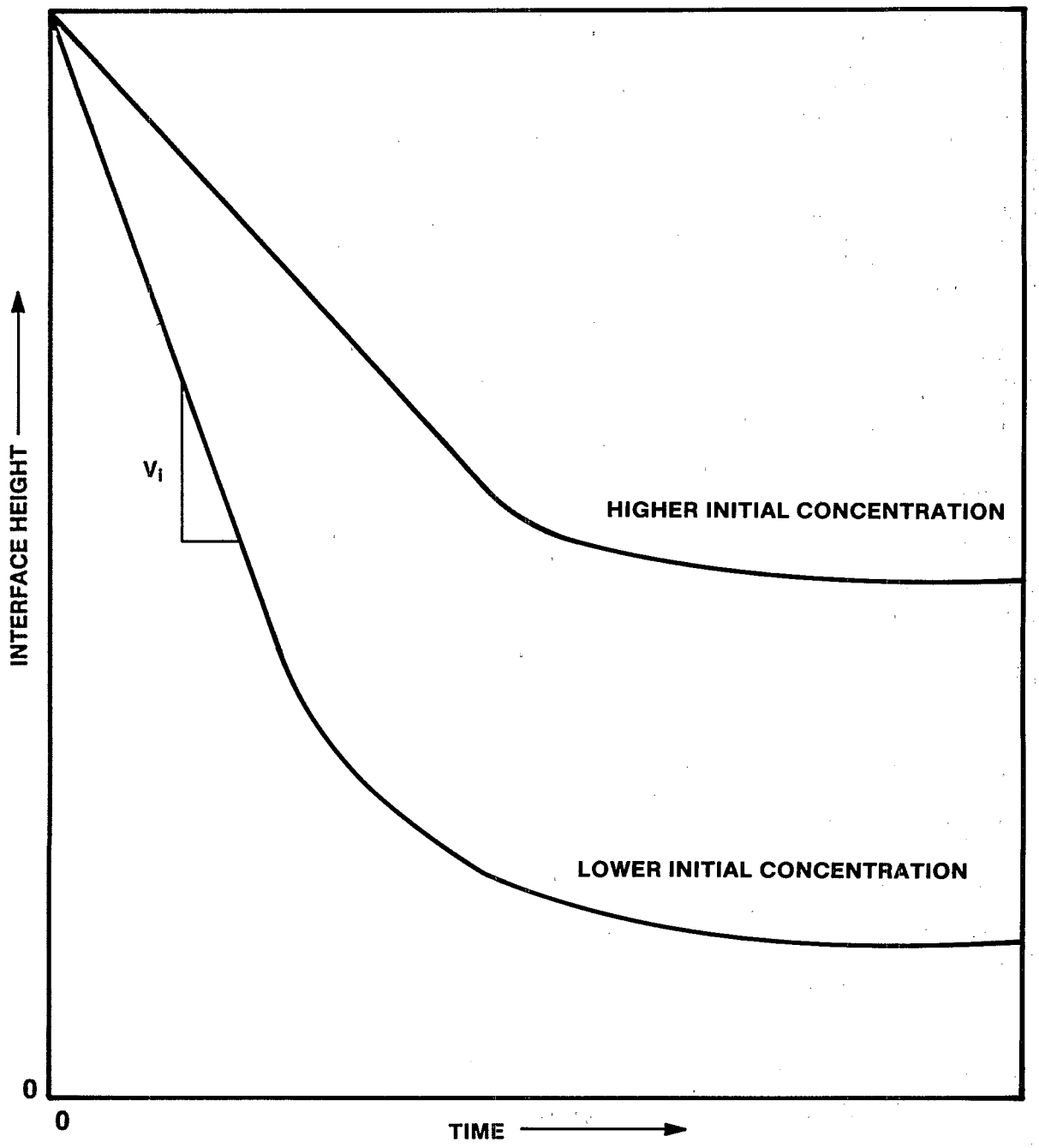
Clarification tank thickening capacity is related to the tank's ability to accept the applied solids load and to convey all the solids to the underflow. This is accomplished in two ways: settling of the activated sludge solids and withdrawal of RAS by pumping. RAS withdrawal causes a general flow of fluid to the bottom of the tank, and this general flow also carries

Figure 5. Activated Sludge Process Schematic.




Reference: Jenkins, *et al.*, 1984.

Figure 6. Zone Settling of Activated Sludge Solids.




Reference: Jenkins, *et al.*, 1984.

the settling activated sludge solids to the bottom where they can be removed.

The various techniques that have been developed to determine the thickening capacity of clarification tanks generally compare the rate at which activated sludge solids are applied to the rate at which they are conveyed to the bottom of the tank by settling and bulk withdrawal. As long as the application rate is no greater than the settling and withdrawal rate, the applied solids will be conveyed to the bottom of the tank and removed (i.e., thickening failure will not occur). If the solids application rate is greater, then excess solids will accumulate in the tank until it is full and solids will overflow into the effluent.

Techniques for clarification tank thickening analysis are relatively complex. They account for SS concentration increases in the clarification tank during settling and the effects of this on the rate at which solids are conveyed to the bottom of the tank by both settling and bulk withdrawal. These techniques are discussed thoroughly in the literature (Keinath et al., 1977); they will not be discussed in detail here.

The thickening capacity of a clarification tank can be stated in terms of an allowable applied solids loading rate ($G_a$), which is the mass of solids applied per unit of clarification tank surface area.

Using the definitions presented earlier, the actual solids loading rate is:

$$G_a = X(Q + Q_r)/A \quad (5)$$

Comparison of the actual solids loading rate with the allowable rate will indicate whether the clarification tank is overloaded and whether it will experience thickening failure.

The allowable solids loading rate is a function of several factors including the RAS flowrate per unit of clarification tank surface area ($Q_r/A$) and the settling characteristics of the sludge. By varying these factors, it may be possible to increase the capacity of the clarification tank and/or of the activated sludge system.

*Clarification Tank Thickening Capacity.* This parameter can be determined by direct measurement or by calculation using the techniques discussed above.

*Direct measurement* of thickening capacity is accomplished by manipulating the clarification tank solids loading until thickening failure occurs. For example, the clarification tank influent flowrate can be increased while the RAS flowrate is maintained constant. The clarification tank sludge blanket depth is monitored and the point at which it begins to increase corresponds to the point at which thickening failure is just beginning to occur. The applied solids loading rate under these conditions is calculated. This is the allowable solids loading rate for the selected RAS rate and the observed sludge settling characteristics. By repeating this procedure at different RAS rates and with sludges of different settling characteristics, the clarification tank thickening capacity can be established for a variety of conditions.

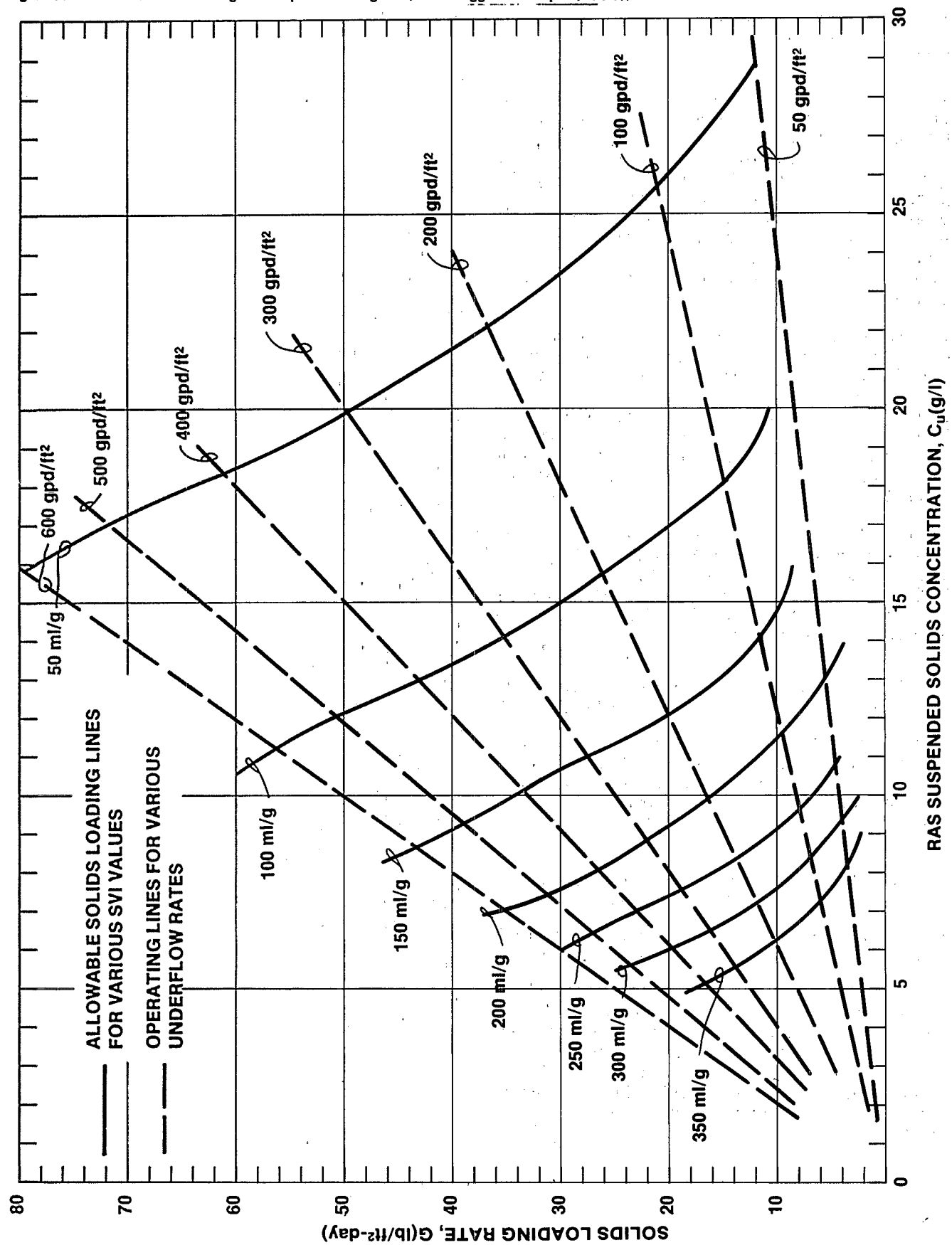
While this technique has been used in practice, it is rather cumbersome and time-consuming, and the effluent quality may be degraded during testing. Moreover, bulking sludge must be available to allow clarification tank capacity to be measured for bulking conditions. The major advantage of this method is that the determination of allowable thickening capacity is direct and no assumptions are required to translate the results to full-scale plant performance.

*Calculation techniques* for determining allowable clarification tank thickening capacity require the measurement of sludge settling characteristics. The relationship between SS concentration and the initial settling velocity must be established either by direct measurement or by using correlations between an index of sludge settleability and the SS concentration/initial settling velocity relationship developed by others. Correlations have been developed using indices such as the standard SVI, the stirred SVI conducted at MLSS = 3.0 g/l, and DSVI.

Direct measurement of the SS concentration/initial settling velocity relationship can be quite cumbersome and time-consuming. Initial settling velocities must be measured for sludges with various initial SS concentrations obtained by mixing various proportions of mixed liquor, RAS, and effluent. Measurements must be made in settling columns that are at least 0.15 m (6 in.) diameter, 1.8 m (6 ft.) tall, and equipped with a stirring device. Bulking sludge must be available before its thickening characteristics can be measured.

Figure 7 presents the results of a correlation technique and analysis developed by Daigger and Roper (1984) in which the allowable solids loading rate ($G_a$) is plotted as a function of the RAS SS concentration for sludges with SVI in the range 50—350 ml/g. Dashed lines that correspond to various underflow rates ($Q_r/A$) also are plotted. This correlation is based on a broad data base and should be readily useable by plant operators because it uses the standard SVI as the index of sludge settleability. To use this diagram, the point that corresponds to clarification tank operating conditions is located in the diagram. Two of the following pieces of information are needed to locate this point:

Figure 7. Clarification Tank Design and Operation Diagram (after Daigger and Roper (1984)).



- The actual solids loading rate;
- The underflow rate; and
- The RAS SS concentration.

The third piece of information can be used as a check. When the point plots below the allowable solids loading line of interest, the clarification tank is operating below its allowable thickening capacity and should not be subject to thickening failure. When the point falls directly on the allowable solids loading line of interest, the clarification tank is operating at the failure point. When the point plots above the allowable solids loading, thickening failure will occur. An example of the use of this diagram is presented later in this section.

*Clarifier Analysis and Operation.* The first steps in clarification tank analysis often are to use the relationships in Equations 1–4. For example, sludge bulking generally will cause a decrease in the RAS SS concentration. Equation 1 predicts that a decrease in the RAS SS concentration ($X_u$) requires an increase in RAS flowrate. This increase is necessary to ensure that sludge applied to the clarification tank is removed in the RAS.

For example, consider an activated sludge system that operates at an influent flow of 1.0 MGD (0.044 $m^3$/sec) and an RAS flow of 0.33 MGD (0.014 $m^3$/sec). The MLSS concentration is 3000 mg/l, and the RAS SS concentration is 12,000 mg/l. As a check, calculate the required RAS flowrate using Equation 1.

$$Q_r = QX/(X_u - X) = (1.0\ MGD)\ (3{,}000)/(12{,}000\text{-}3{,}000)$$
$$= 0.33\ MGD\ (0.014^3/sec)$$

This agrees with the actual RAS flowrate. Now assume that the sludge settleability deteriorates and that the clarifier sludge blanket begins to increase. The RAS SS concentration is found to be 8,000 mg/l. Equation 1 indicates that the RAS flowrate must be:

$$Q_r = QX/(X_u\text{-}X) = (1.0\ MGD)\ (3{,}000)/(8{,}000\text{-}3{,}000)$$
$$= 0.60\ MGD\ (0.026 m^3/sec)$$

Thus, for these conditions increasing the RAS flowrate to 0.60 MGD should prevent clarification tank failure.

The use of Equations 1–4 to develop alternative clarification tank operating strategies must be accompanied by an analysis of clarification tank thickening capacity. In this manual, the correlation method (with SVI) of Daigger and Roper (1984) will be used; as indicated previously, other somewhat less convenient techniques are available.

Assume that the clarification tank in the example discussed above is 45 feet (13.7 m) in diameter and has a surface area of 1,590 $ft^2$ (148 $m^2$). From Equation 5, the applied solids loading rate for the intitial operating condition is:

$$G_a = X(Q + Q_r)/A$$
$$= (3{,}000\ mg/l)(1.0 + 0.33)MGD(8.34)/1{,}590\ ft^2$$
$$= 20.9\ lb/ft^2\text{-}day\ (102\ kg/m^2\ day)$$

This condition plots as point number 1 in Figure 41 and indicates that the clarification tank could operate successfully with a sludge having an SVI of up to 150 ml/g.

For the second operating condition (RAS, SS = 8,000 mg/l, RAS flowrate = 0.6 MGD) the applied solids loading rate is:

$$(3{,}000\ mg/l)(1.0 + 0.6)MGD(8.34)/1{,}590\ ft^2$$
$$= 25.2\ lb/ft^2\text{-}day\ (123\ kg/m^2\ day)$$

This condition plots as point number 2 in Figure 41 and indicates that the clarification tank now can operate successfully with a sludge having an SVI just over 200 ml/g.

Now assume that the maximum RAS pumping capacity is 0.95 MGD (0.042 $m^3$/sec). At the maximum RAS flowrate, the bulk withdrawal rate is 0.95 MGD/1,590 $ft^2$ or 597 gpd/$ft^2$ (24.3 m/day) and the applied solids loading rate is:

$$(3{,}000\ mg/l)(1.0 + 0.95(MGD(8.34)/1{,}590\ ft^2$$
$$= 30.7\ lb/ft^2\text{-}day\ (150\ kg/m^2\text{-}day)$$

This condition plots as point number 3 on Figure 41 and indicates that the clarification tank could be operated successfully with a sludge having an SVI of about 250 ml/g and that the RAS SS concentration would be about 6,300 mg/l.

Now assume that the RAS flowrate is maintained at 0.95 MGD (0.042 $m^3$/sec) (equivalent to a bulk withdrawal rate of 597 gpd/$ft^2$) (24.3 m/day) but that the SVI will be controlled to a value of no more than 150 ml/g by RAS chlorination. Moving along the operating line (for 597 gpd/$ft^2$) to point number 4 indicates that the clarification tank could be operated at a solids loading rate of up to 42.8 lb/$ft^2$-day (209 kg/$m^2$-day) and with an RAS SS concentration of up to 8,700 mg/l at this loading.

Equation 5 can be arranged to calculate for these conditions:

a. The maximum allowable influent flow rate at MLSS = 3000 mg/l

$$Q = (G_a A/X) - Q_r$$

$$= (43.5 \text{ lb/ft}^2\text{-day}(1{,}590 \text{ ft}^2)/(3{,}000 \text{ mg/l})(8.34)) - 0.95 \text{ MGD}$$

$$= 1.8 \text{ MGD } (0.79 \text{ m}^3/\text{sec})$$

b. The maximum allowable MLSS at an influent flow of 1.0 MGD (0.079 $m^3$/sec.)

$$X = (G_a A)/(Q + Q_r)$$

$$= (43.5 \text{ lb/ft}^2\text{-day})(1{,}590 \text{ ft}^2) \div (1.0 + 0.95)\text{MGD}(8.34)$$

$$= 4{,}250 \text{ mg/l}$$

These examples illustrate how the general principles of clarification tank analysis can be used to optimize the existing activated sludge clarification tank operation.

*System Analysis and Operation.* In addition to manipulation of RAS flowrates, the effects of bulking sludge can be ameliorated by reducing MLSS concentration in the clarification tank feed. This reduces the applied solids loading rate to the clarification tank (Equation 5). Reducing applied solids loading rate while maintaining the same RAS bulk withdrawal rate (i.e., move downward and to the left along an RAS bulk withdrawal operating line in Figure 8) increases the allowable SVI.

MLSS concentration in the clarification tank feed can be reduced by reducing the mixed liquor solids inventory and by changing the activated sludge operating mode. Mixed liquor solids inventory can be reduced by increasing the sludge wasting rate. This may not be feasible if sludge handling capacity is not available or if reducing the mixed liquor solids inventory would lead to an unfavorable F/M (e.g., a low F/M may be required to achieve nitrification).

The objective of changing operational mode to combat sludge bulking is to reduce MLSS concentration in the clarification tank feed without reducing the mixed liquor solids inventory. The step feed (step aeration) and contact stabilization configurations are particularly useful in this regard. Needless to say, they can only be employed to combat bulking sludge if the plant is designed with the flexibility to operate in these configurations. Both operating configurations allow the operation of part of the aeration tank at a higher MLSS concentration (for a given F/M) than would be acheived were the aeration tank completely mixed.

The use of step feed to lower the solids loading applied to the clarification tank is illustrated using the previous example. In addition to an influent flowrate (Q) of 1.0 MGD (0.044 $m^3$/sec), an RAS flowrate of ($Q_r$) of 0.33 MGD (0.014 $m^3$/sec), an MLSS concentration (X) of 3,000 mg/l, an RAS concentration ($X_u$) of 12,000 mg/l, and a clarification tank solids loading rate of 20.9 lb/ft²/day (102 kg/m²/day), it is assumed that the aeration tank has a total volume of 0.25 MG (3780 $m^3$) and that it can be operated in either the plug flow (conventional) or the two-pass step feed mode (Figure 9).

Point number 1 in Figure 10 indicates that these operating conditions are acceptable when the SVI is less than 150 ml/g.

When the operating mode is changed to two-pass step feed at the same influent and RAS flow rates, all of the RAS but only one-half of the influent flow is added to the first pass. The remainder of the influent flow is added to the second pass. A redistribution of mixed liquor solids inventory occurs so that it is higher in the first pass than in the second pass. This arises because less dilution of the RAS by influent occurs in the first pass than in the second pass. If the total mixed liquor inventory is maintained the same as in the plug flow mode, then the MLSS concentration in the second pass will be less than when the system was operated in the plug flow mode.

The MLSS concentrations in each of the two passes can be calculated for step feed by writing solids mass balances for each pass. These equations then are substituted into an equation for the total solids inventory, which is solved for the solids concentrations in each pass and in the RAS. For our example, the aeration tank MLSS inventory in the plug flow mode is:

$$\text{Aeration Tank Inventory} = (3{,}000 \text{ mg/l})(0.25 \text{ MG})(8.34)$$

$$= 6255 \text{ lb } (2840 \text{ kg})$$

This inventory will be maintained in the two-pass step feed mode.

The solids mass balance for the point at which the influent flow to the first pass (0.5 MGD) is mixed with the RAS flow (0.33 MGD) is:

$$(0.33 \text{ MGD})(Xu) = (0.5 + 0.33 \text{ MGD})(X_1)$$

and

$$X_1 = (0.33/0.833)X_u = 0.4\,X_u \qquad (6)$$

Figure 8. Clarification Tank Thickening Analysis Example (after Daigger and Roper (1984) ).

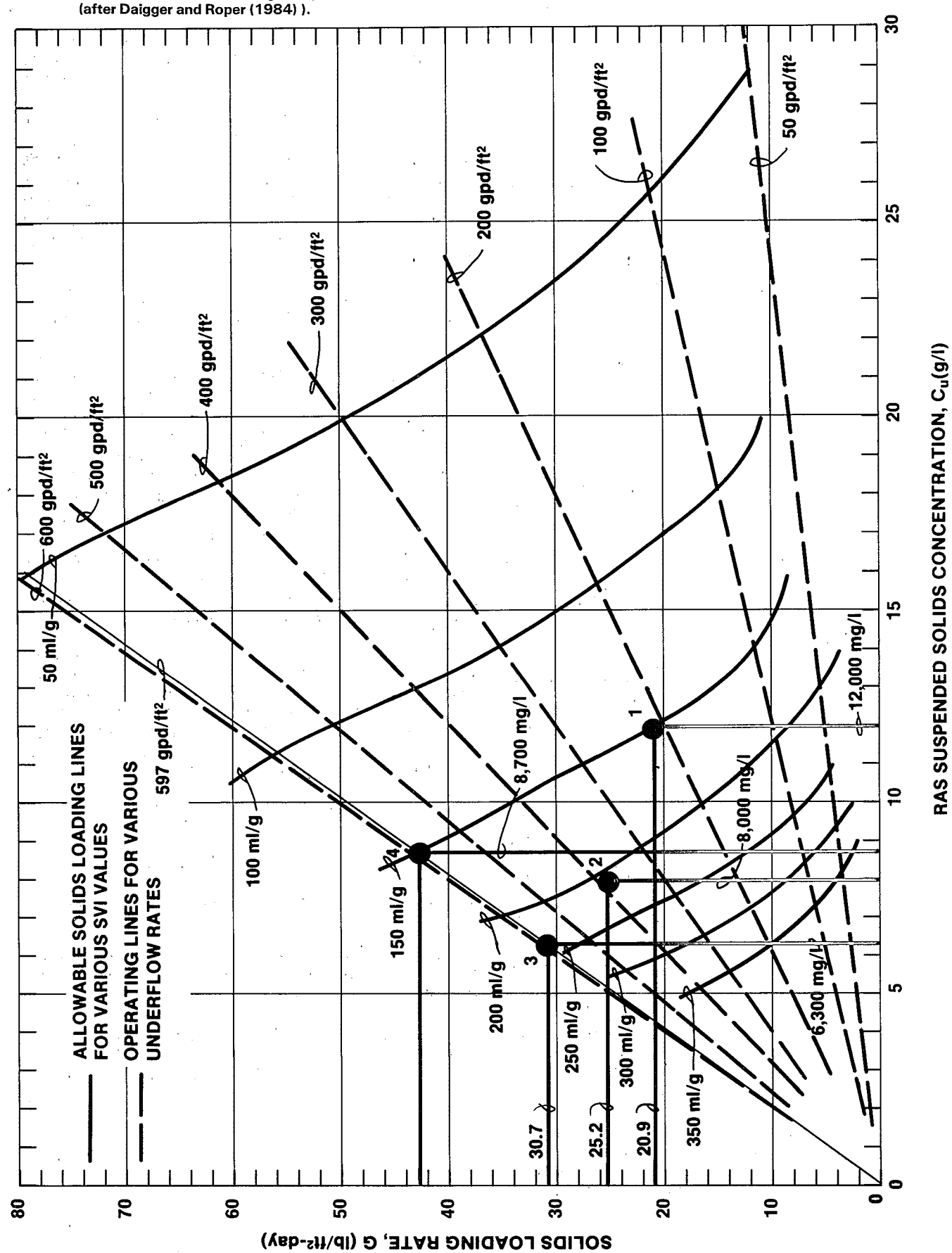
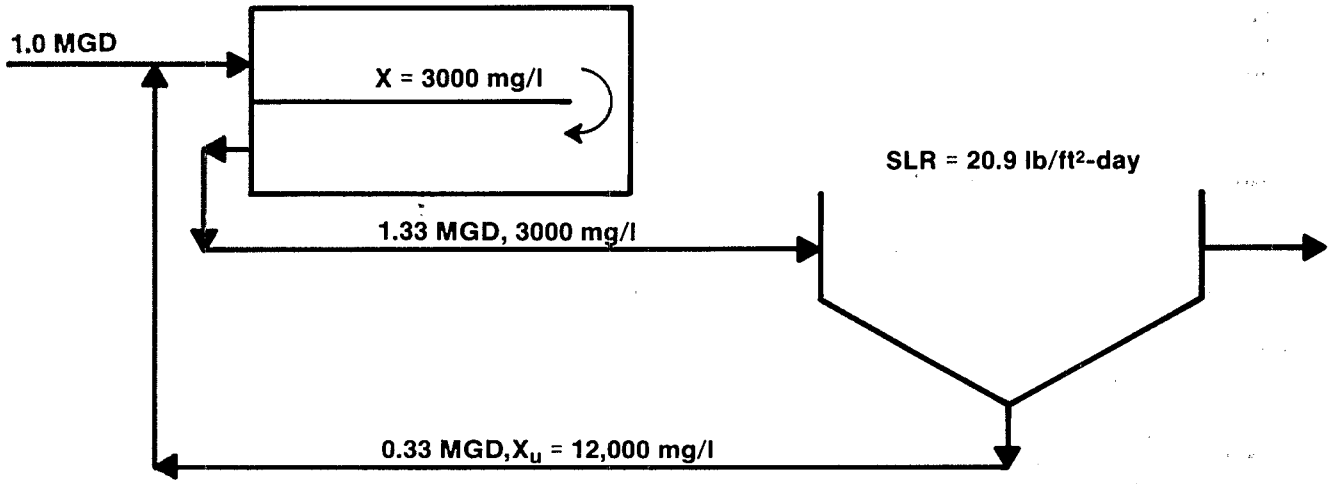
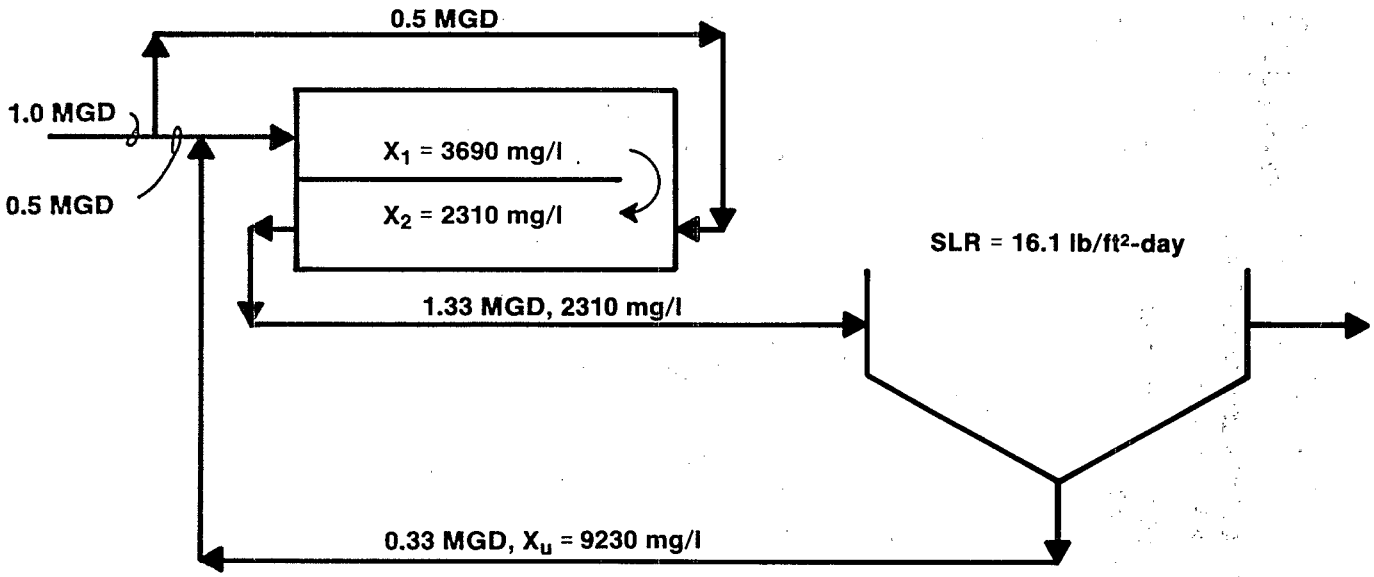


Figure 9. Step Feed Operation Example Schematic
(After Daigger and Roper (1984) ).

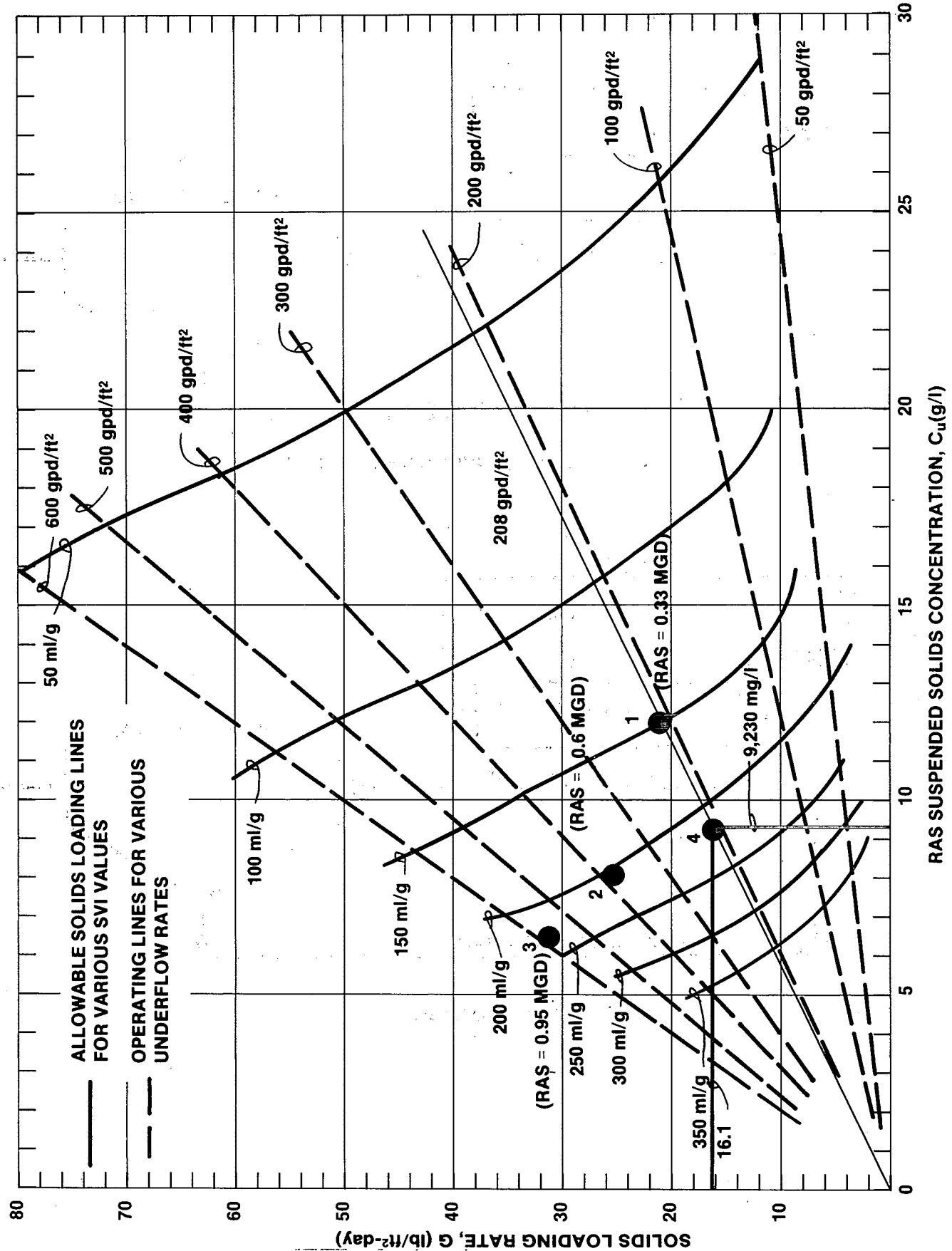



PLUG FLOW (CONVENTIONAL) MODE




STEP FEED MODE

Figure 10. Step Feed Operation Example Plot of Parameters (after Daigger and Roper (1984)).



The solids mass balance for the point at which the flow from the first pass (0.83 MGD) mixes with the influent flow to the second pass (0.5 MGD) is:

$$(0.83 \text{ MGD})(X_1) = (0.83 + 0.5 \text{ MGD})(X_2)$$

and

$$X_2 = (0.83/1.33)X_1 = 0.625X_1 \quad (7)$$

Combining Equations 6 and 7 gives:

$$X_2 = 0.625X_1 = 0.625(0.4X_u) = 0.25X_u \quad (8)$$

The total aeration tank mixed liquor solids inventory can be expressed as a function of $X_u$ as follows, remembering that the volume of each aeration tank pass is 0.25 MG/2 or 0.125 MG (1890 $m^3$):

Aeration Tank Solids Inventory =

$$(X_1)(0.125 \text{ MG})(8.34) + (X_2)(0.125 \text{ MG})(8.34) = 1.0425\,(X_1 + X_2) \quad (9)$$

Substituting for $X_1$ and $X_2$ from Equations 6 and 8 gives:

Aeration Tank Solids Inventory =

$$1.0425(0.4X_u + 0.25X_u)$$

$$= 0.6776X_u$$

Setting this equal to the total solids inventory of 6255 lb gives:

$$6255 \text{ lb} = 0.6776X_u$$

and

$$X_u = 9{,}230 \text{ mg/l}$$

$X_1$ can be calculated using Equation 6:

$$X_1 = 0.4X_u = (0.25)(9{,}230 \text{ mg/l}) = 3{,}690 \text{ mg/l}$$

and $X_2$ can be calculated using Equation 8:

$$X_2 = 0.25X_u = (0.25)(9{,}230 \text{ mg/l}) = 2{,}210 \text{ mg/l}$$

The clarification tank solids loading rate for this operating condition would be:

$G_s = (2{,}310 \text{ mg/l})(1.33 \text{ MGD})(8.34/1{,}590 \text{ ft}^2$
$= 16.1 \text{ lb/ft}^2\text{-day}$ (78.6 kg/$m^2$-day) (see Figure 10)

The benefits of two-pass step feed operation in allowing an activated sludge system to operate successfully with a poor settling sludge are illustrated in Figure 10 using the data from the previously calculated examples. Points numbers 1 through 3 are for plug flow operation with RAS flowrates of 0.33, 0.6, and 0.95 MGD (0.01, 0.026, and 0.04 $m^3$/sec), respectively. Point number 4 is for two-pass step feed operation at an RAS flowrate of 0.33 MGD (0.01 $m^3$/sec), as calculated above. These operating points show that a sludge with an SVI of approximately 220–230 ml/g requires an RAS flowrate of 0.95 MGD when the aeration tank is plug flow and 0.33 MGD (0.01 $m^3$/sec) when the aeration tank is two-pass step feed. This results both in lower RAS pumping costs and higher RAS, SS concentration (9230 mg/l *vs.* 6300 mg/l)—which may reduce waste sludge processing and disposal costs.

This example illustrates the advantage of two-pass step feed over plug flow operation for dealing with bulking sludge. Operation in other step feed modes (such as three- or four-pass), contact stabilization, or step feed with RAS reaeration (a combination of step feed and contact stabilization) offer similar advantages. Indeed, any change which results in less dilution of RAS by influent wastewater will allow storage of activated sludge solids in the aeration tank and a decrease in the MLSS concentration in the clarification tank feed. For example, if the system depicted in Figure 42 had been placed in the contact stabilization mode, using the first pass for sludge stabilization and the second pass for contact (while maintaining the same mixed liquor solids and inventory), the MLSS concentration in the clarification tank feed would have been reduced to 1,200 mg/l and the clarification tank solids loading rate would be 3.4 lb/$ft^2$/day (41 kg/$m^2$/day).

These examples have illustrated calculation procedures for determining the operating conditions when the mode of operation is changed. In some cases a simplified calculation procedure can be developed for a particular system operating under defined operating conditions, or operating diagrams can be developed for a particular system. Alternatively, these concepts can be used in a plant by changing plant operation and observing the results. The general principle that changes resulting in less dilution of RAS by influent wastewater will decrease the MLSS concentration of the clarification tank feed can be used to guide changes in operating mode. After making changes, their impacts on solids distribution in the system can be monitored. In most cases redistribution of the solids takes less than 1 day, so that the results can be determined in a timely manner. The resulting clarification tank solids loadings can then be determined and the need for further changes assessed.

### *3.4.3 Addition of chemicals to enhance settling.*

The intent of this technique is to improve settling of sludge without destroying the filamentous microorganisms. Synthetic polymers, added to the mixed liquor as it leaves the aeration tank or to the clarification tank entry point, aid in settling of diffuse filamentous floc. The polymer used is usually a high molecular weight, cationic charge polymer, and may be applied alone or

**Table 3. Bulking Filamentous Microorganisms That Have Been Successfully Controlled by Chlorination**

| Location | Major Filamentous Organism(s) Causing Bulking |
|---|---|
| City of Albany, GA | type 0041<br>type 0092<br>*H. hydrossis*<br>type 1701 |
| Malibu Mesa, CA | type 1701<br>type 021N |
| City of Los Angeles Terminal Island, CA | type 021N<br>type 0581 |
| Carrousel Pilot Plant Brewery Waste | type 0041<br>type 0092 |
| City of Pacifica, CA | *S. natans*<br>type 1701<br>type 1863 |
| City of San Jose, CA | *H. hydrossis*<br>type 1701<br>type 021N<br>type 0812<br>type 0041<br>type 0675 |
| Derry Township Municipal Authority, Hershey, PA | *Thiothrix sp*<br>*M. parvicella* |
| Ormo Loma Sanitary District, CA | *H. hydrossis*<br>type 1701 |
| Weyerhaeuser Longview, WA (NaOCl) | type 0675<br>type 1851 |
| Miller Brewing Co. Fulton, NY | type 0041<br>type 0675 |
| Stroh Brewing Co. Longview, TX (NaOCl to Aeration Basin) | type 1851<br>type 021N<br>*Nostocoida limicola II* |
| Champion International (Pulp and Paper) Courtland, AL | *Thiothrix sp*<br>type 1701 |
| Monterey Regional Water Pollution Control Plant Monterey, CA | *Nostocoida limicola II*<br>type 1701<br>*Thiothrix sp* |
| City of Redding, CA | type 1701<br>*S. natans* |
| Westchester County Yonkers, NY | type 1701<br>*Nostocoida limicola* |
| Thames Water Authority (UK) (NaOCl) | type 021N |
| Central Contra Costa Sanitary District Concord, CA | type 021N<br>*M. parvicella*<br>*Thiothrix sp* |
| City of Pacifica, CA | *S. natans*<br>type 1701 |

in combination with an anionic polymer. In some cases, lime, ferric chloride, or other inorganic precipitants have been added to systems, producing a heavy precipitate that forces the sludge floc to settle. This method greatly increases the solids load the system must bear.

Because overdosing with polymers can degrade system performance, careful testing must be done to estimate appropriate doses.

The use of polymers is an expensive method of bulking control. Chemical costs of up to $450/MG have been reported.

### 3.4.4 Addition of chemicals to selectively kill filamentous microorganisms.

Two main chemicals—chlorine and hydrogen peroxide—have been successfully used in the selective destruction of filamentous microorganisms.

#### 3.4.4.1 Chlorine.

The use of chlorine is popular in part because it is frequently used in effluent disinfection, and is thus available at most activated sludge plants. Chlorine may be added at three points in the system: directly into the aeration tank; into a ''loop'' in which mixed liquor is withdrawn from the aeration tank, chlorinated, and returned to the tank; or into the return activated sludge (RAS) stream. The last is the most common and preferred choice, except in plants where aeration time is very long or where the RAS line is inaccessible.

The following guidelines must be followed for successful use of chlorination:

1) A target value of SVI or some other floc parameter must be set.

2) Chlorination must be used only when the target value is regularly exceeded; a careful, frequent plot of the parameter should be made to determine whether it consistently exceeds the target value.

3) Chlorine doses must be known and carefully controlled, and must be added to the sludge at a point where it will mix efficiently. If it does not mix well, parts of the sludge will overdose while parts will not be chlorinated at all. Poor mixing will cause turbid effluents, reduction in treatment efficiency due to killing of floc-forming organisms, and consumption of large amounts of chlorine without control of the bulking problem.

4) Chlorine should be added where chlorine demand is at minimum—that is, where the amount of organic matter is lowest. Chlorine reacts quickly with ammonia and organic matter, and becomes unavailable for killing filamentous microorganisms. It should not

be added to influent wastewater, or to RAS after it has begun to mix with influent waste just outside the aeration tank.

5) Appropriate parameters must be considered in selecting a dosing point. These are:

Overall Mass Dose Expressed in $\frac{\text{kg } Cl_2}{10^3 \text{ kg SS}}$ per day

Local Mass Dose Expressed in $\frac{\text{kg } Cl_2}{10^3 \text{ kg SS}}$ per day

Frequency of Exposure $\text{day}^{-1}$ (times per day sludge passes point of dose)

Overall Mass Dose is based on the plant's total sludge inventory and tells how much chlorine must be added per day. However, it does not indicate how often and in what concentration the chlorine should be applied for a given application point in the system. The other three parameters provide such information.

The local mass dose indicates the quantity of chlorine which must be added at a dosing point in order to control the filamentous microorganisms. The dose concentration is the concentration of chlorine to be added at the dosing point to achieve the local mass dose. However, the dose concentration must be low enough so that a small part of the sludge inventory is not exposed to excessively high chlorine doses. In such cases, the exposed sludge floc can be completely destroyed while the bulk of the solids inventory is unaffected. Also, the frequency of exposure of the sludge inventory to the chlorine dose must be high enough to control the filamentous microorganisms (generally greater than once per day). If proper control of filamentous microorganisms cannot be achieved because of insufficient frequency of exposure or because the local mass dose cannot be achieved at a reasonable dose concentration, then a change in the dosing point or the addition of a dosing point(s) may be necessary.

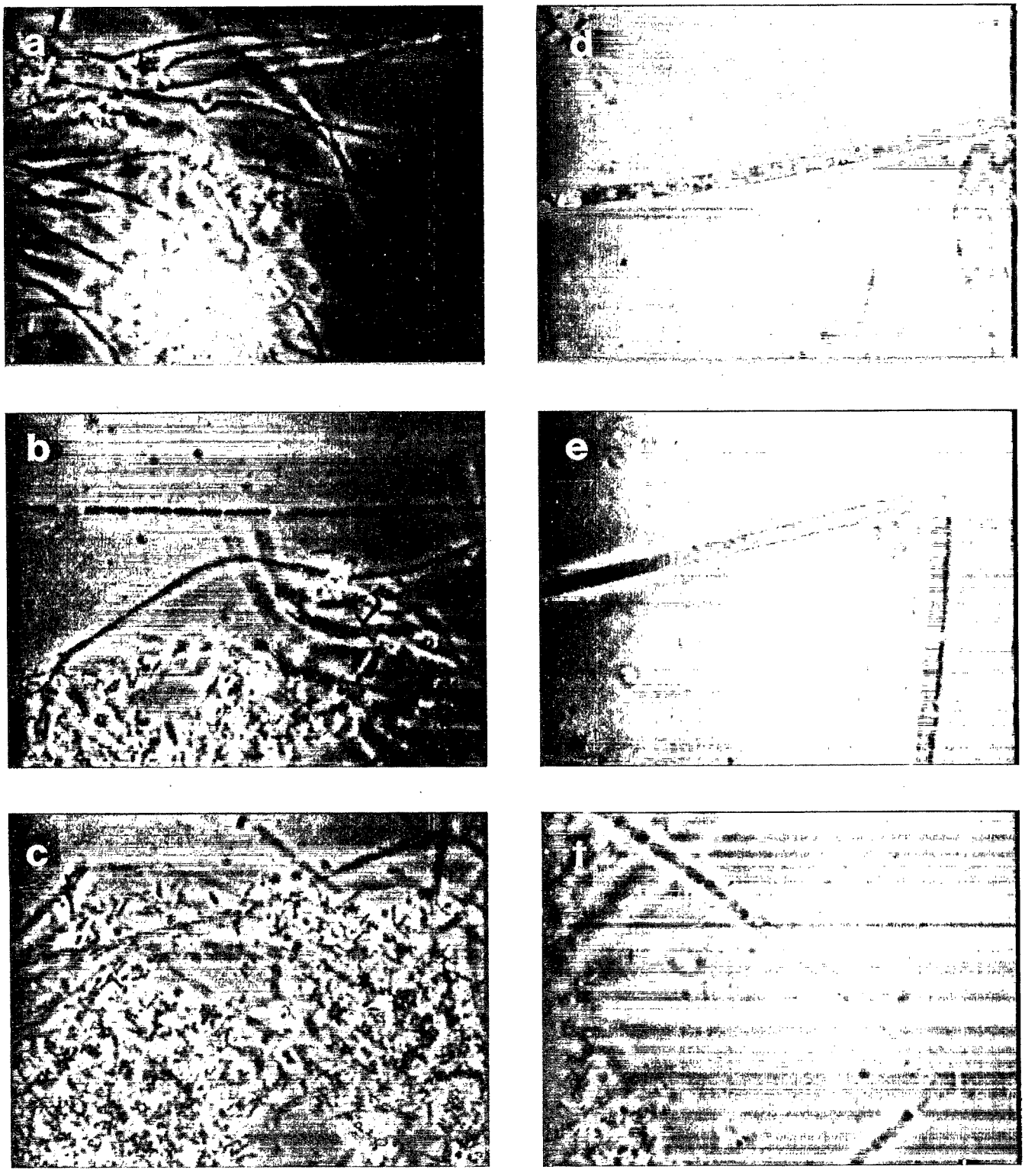
6) Reliable control tests must be performed to assess the effectiveness of chlorination. These include a measure of sludge settling rate (such as SVI); a measure of final effluent turbidity (such as a turbidimeter or a Secchi disc); and microscopic examination of the sludge, to observe progressive deformation of filamentous microorganisms or to detect signs of overdosing (such as total elimination of filamentous microorganisms and presence of broken flocs). The progressive effects of chlorination on type 1701 and *Thiothrix* spp. are illustrated in Figure 11. Types of filamentous microrganism successfully controlled by chlorination appear in Table 3.

**Table 4. Use of Hydrogen Peroxide for Bulking Control**

| Location | Reference | Successful concentration, mg/l |
|---|---|---|
| PETALUMA<br>full-scale plant | ANON, FMC Corp. (1973)<br>Caropreso *et al.* (1974) | 9–68<br>average 31.5 |
| ST. AUGUSTINE<br>full-scale plant | ANON, FMC Corp. (1976) | 12 (basis unclear) |
| SAN JOSE<br>lab scale,<br>fill and draw | Strunk and Shapiro (1976) | 40–200 |
| PRINCETON<br>small full-scale | Caropreso *et al.* (1974) | 100[b] |
| WILMINGTON<br>lab-scale | Cole *et al.* (1973) | 40–200[b] |
| WASHINGTON<br>pilot plant | Cole *et al.* (1973) | 20–40[b]<br>200–400[a] |
| TEXTILE MILL | Keller and Cole (1973) | 60 (basis unclear) |

[a]Based on wastewater inflow.
[b]Based on volume of aeration basin and clarifier (single dose).

Figure 11. Progressive effects of chlorination on type 1701 (*a, b,* and *c*) and *Thiothrix* sp. (*d, e,* and *f*): *a.* and *d.* no chlorination; *b.* and *e.* moderate chlorination effects; and *c.* and *f.* severe chlorination effects (all 1000X phase contrast).




Reference: Jenkins *et al.*

### 3.4.4.2 Hydrogen peroxide.

The use of hydrogen peroxide ($H_2O_2$, 50 percent by volume) has been effective in both continuous and batch dosing when added to the aeration tank, to the RAS line, and to the mixed liquor as it passes between the aeration tank and the clarification tank. It presumably attacks the sheath of the filamentous microorganism, destroying its shape, and resulting in progressive deformation like that seen in chlorination.

Mixing is important with $H_2O_2$ as it is with chlorine. Somewhat higher doses and longer contact times may be needed.

It is possible that as $H_2O_2$ kills filamentous microorganisms, it also releases oxygen. If bulking was a result of low DO, this could provide additional improvement in operations. However, if sludge oxidizes $H_2O_2$ before it has a chance to kill filamentous microorganisms, this treatment will not be effective.

Examples of the use of hydrogen peroxide in bulking control are shown in Table 4.

## Chapter 4

## 4.0 Activated Sludge Foaming

Several different types of foam have been observed in activated sludge treatment systems. Some are normal and harmless, while others are signs of operating problems and need to be controlled.

## 4.1 Symptoms of Sludge Foaming

At the startup of an activated sludge plant, while solids concentrations and Sludge Age are low, white froth usually occurs. As the process stabilizes and solids build up, this type of foam usually disappears. If excessive, sudsy white foam persists, the Sludge Age may be too low, or there may be nonbiodegradable substances in the waste (industrial wastes or cleaners). A more troublesome foam, heavy, viscous, and brown, can appear on the surface of both aeration and clarification tanks, degrading the effluent and spilling over onto walkways. While this type of foam has been attributed to a high Sludge Age, filamentous microorganisms may also be involved in its production.

## 4.2 Role of Filamentous Microorganisms in Sludge Foaming

Analysis of samples of sludge from systems containing heavy brown foam has revealed large numbers of the filamentous microorganism *Nocardia* (see Figure 12). While the mechanisms of *Nocardia* foaming are not fully understood, it is believed that *Nocardia* cell walls are hydrophobic, and sufficient quantities of *Nocardia* can make sludge flocs hydrophobic. Thus the floc would not be wettable and would cling to air bubbles, resulting in a scum.

## 4.3 Factors Affecting the Presence of *Nocardia*

The causes of *Nocardia* growth in activated sludge are not well understood. However, researchers have found correlations between high *Nocardia* counts and various sludge parameters. These include high Sludge Age and warm temperature oil and grease in the wastewater, low F/M ratio, and high SS levels.

## 4.4 Control of Activated Sludge Foaming

Because the mechanisms of this phenomenon are as yet so little understood, there is no single proven method of controlling it. However, some standard methods have had some success.

### *4.4.1 Physical methods.*

The most common physical method of foam control is the use of low-volume water spray to rupture the bubbles in the foam. This method is not effective with very stable foams, which usually must be collapsed and diluted by high-volume sprays. Use of this method is often complicated by the fact that the scum traps in most secondary clarifiers are too small to receive the amount of foam *Nocardia* can cause, and the scum trap drainage pipes are usually too narrow for such foam to flow out.

Grease and oil related problems are best solved by a strong industrial waste enforcement program and control of commercial grease traps. High amounts of grease and oil coupled with high temperatures and high suspended solids contribute to the presence of *Nocardia* and may act as a causitive agent to keep the population alive.

### *4.4.2 Process manipulations.*

The most common approach to *Nocardia* foaming control is to reduce the Sludge Age by increasing the sludge wasting rate, thus washing out the *Nocardia*, and concurrently increasing the F/M loading. This process is affected by temperature—the higher the waste temperature, the further the operator must lower the Sludge Age. This approach works only when the plant is capable of handling the increase in waste activated sludge, and when nitrification will not be performed (the Sludge Age required for nitrification makes *Nocardia* washout impossible by this means).

Another process manipulation involves the addition of sludge from an anaerobic digester. Such sludge may contain material that is toxic to *Nocardia*. This method has had only mixed success in laboratory studies.

### *4.4.3 Chemical methods.*

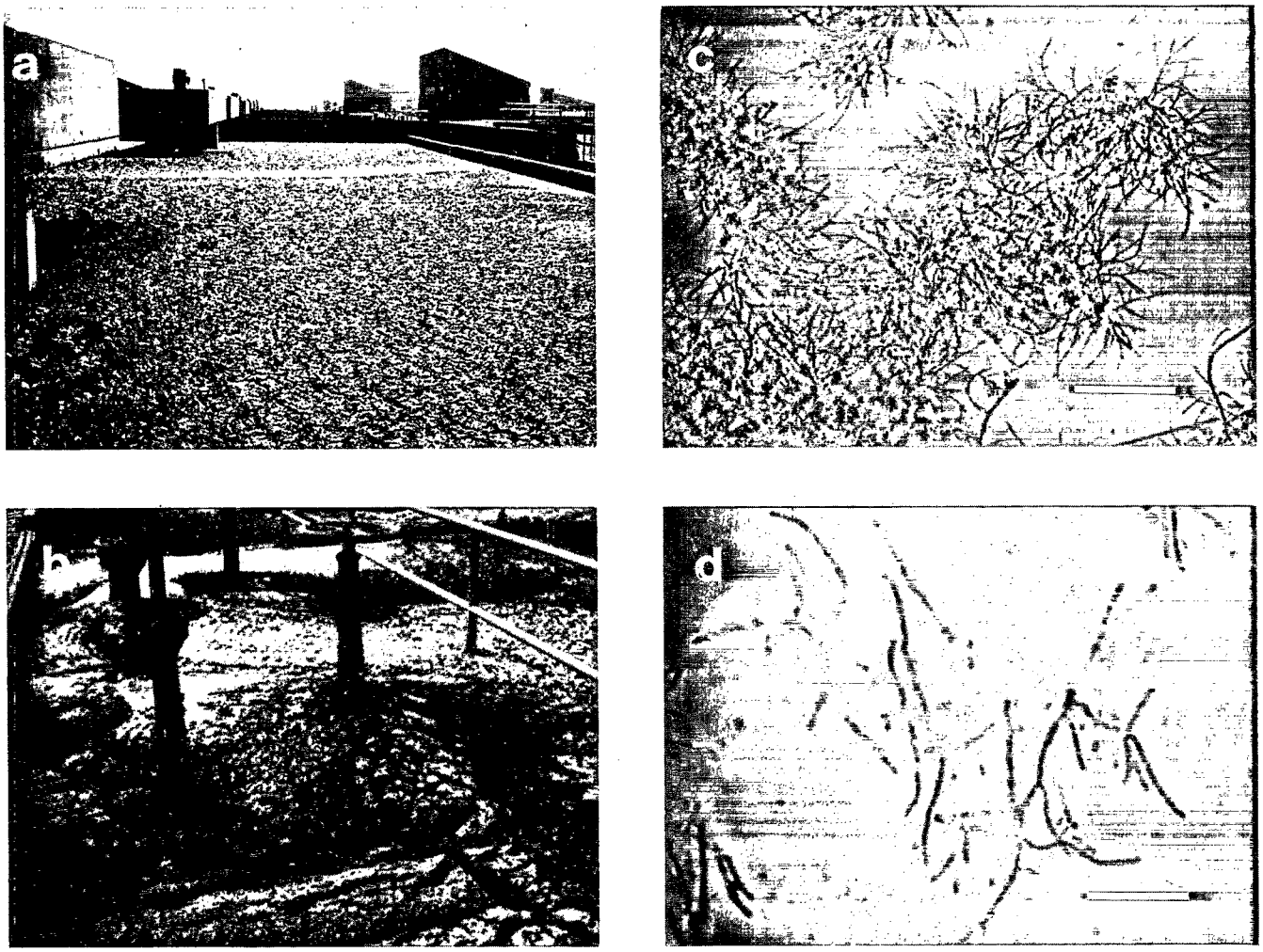
The use of chemical antifoam agents in combatting *Nocardia* foam does not have a good record of success.

However, a combination of physical removal, chlorination, and addition of ferric chloride has been

used to reduce *Nocardia* populations to a manageable level.

The most important consideration in control of *Nocardia*, regardless of the method chosen, is the prevention of foam recycling. If such foam is recycled through the system, it will reseed the sludge. When the contents of scum traps are fed back to gravity thickeners or the RAS, while wasting continues to remove the mixed liquor and sludge from which the scum separated, *Nocardia* becomes concentrated in the system.

Figure 12. *Nocardia* foaming in activated sludge: *a.* and *b.* foam on the aeration basin; *c.* and *d.* microscopic appearance of Nocardia foam (*c.* 400× phase contrast; bar = 25 μm; *d.* 1000× phase contrast; bar = 10 μm).



## Chapter 5

## 5.0 Appendix 1—Methods for Microscopic Examination of Filamentous Microorganisms in Activated Sludge

Microscopic examination of activated sludge is an important tool for the identification of sludge settling problems. Determining the number and type of filamentous microorganisms is the first step in controlling bulking and foaming.

Such examination requires a research-grade phase contrast microscope with 100× and 900–1000× phase contrast objectives. A mechanical stage is essential for controlled scanning. A binocular head (or trinocular head if photography is desired) and built-in light source are highly recommended. An ocular micrometer should be inserted into one of the eyepieces and calibrated at each magnification employed using a stage micrometer.

A photographic record of sample appearance at 100× is recommended for reference purposes. A 35mm camera mounted on the trinocular head is most convenient. The camera should have a built-in light meter and a replaçeable, fine ground-glass focusing screen. Polaroid instant film cameras can be used. It is convenient and highly efficient to take all photographs using color slide film (e.g., Kodak tungsten-balanced type II professional film designated KPA), and produce color or black-and-white prints from the slides using a slide printer employing Polaroid instant film.

Because many of the observations to be made in these procedures are close to the limit of resolution of the light microscope, and many of the features to be studied are difficult to detect, the microscope must be in good condition. Regular adjustment of the phase rings is required, the microscope objectives must be kept clean, and a dust-free environment is necessary. Professional servicing of the microscope by the manufacturer or recommended agent should be performed at least once per year.

### 5.1 Sampling Methods

Samples of activated sludge mixed liquor should be taken from the effluent end of the aeration tank, or from the mixed liquor channel between the aeration tank and the clarification tank. Mixed liquor samples should be taken below the surface, excluding any foam or other floating material. If the activated sludge is foaming, a separate foam sample should be taken from the surface of the effluent end of the aeration tank, from the surface of the mixed liquor channel, or from the surface of the clarification tank. Subsurface liquid should be excluded from foam samples. Although foams can be thick and viscous and difficult to transfer to sample bottles, they should not be diluted to ease sampling, because this will prevent comparison of the relative abundance of filamentous microorganisms in the foam and the mixed liquor.

Some activated sludge process modifications contain more than one tank, e.g., step-feed, contact stabilization, or "plug-flow" systems. In such systems, characteristics of the sludge in all tanks are usually similar. Thus it is usually not necessary to sample all tanks, only the effluent end of the aeration tank. Where an activated sludge plant consists of separate and parallel systems, there may be differences in floc and filamentous microorganism characteristics in each system, especially if there is not complete admixture of the return activated sludge (RAS) streams. In this situation, a sample is required from each system. Similarly, for two-stage activated sludge systems (e.g., "carbonaceous" first-stage followed by "nitrification" second-stage), a sample from the aeration tank of each stage is necessary for proper characterization of floc structure and filamentous microorganism populations. Some activated sludge systems treat a waste that has been pretreated in another type of biological treatment system (lagoon or trickling filter). These units may seed the activated sludge with filamentous microorganisms. To determine the extent of this seeding, a sample should be taken of the waste entering the activated sludge system.

Sampling and examination frequency will be dictated by plant circumstances and by the location of examination of samples. During critical periods (when bulking is occurring or is anticipated, during the use of RAS chlorination for bulking control and during periods of experimental operation), daily onsite examination can be justified. Routine onsite examination may be performed once or twice per week. Offsite

examination may be performed weekly, monthly, or seasonally, depending on the severity of problems being encountered or the desire to establish an operating history (and on the budget available for this activity).

Samples should be examined as soon as possible after they are taken. When examination is performed onsite, samples not examined within several hours should be stored at 4°C in a refrigerator. When samples must be transported offsite for analysis, they should be sent in sample containers in which there is an air space at least equal to the volume of the sample, to avoid septicity. (Five to 10 ml are needed for typical microscopic examination, so a 20–25 ml vial will be adequate.) The sample should be neither chemically preserved nor frozen, since these procedures can alter the characteristics of the flocs and filamentous microorganisms. The longer the time that elapses between sampling and examination, the more difficult and uncertain sample examination and data interpretation become. Samples from plants with low organic loadings (long Sludge Ages) maintain their characteristics longer than samples from plants with high organic loadings (short Sludge Ages). For long Sludge Age samples, a satisfactory examination can be obtained if the sample is looked at within 7 to 10 days of sampling; for sludges from highly loaded plants, it is wise to examine the activated sludge within 3 to 4 days of its sampling. With the availability of express mail and overnight delivery services, transit time should pose no problem in offsite analysis.

Filamentous microorganism staining reactions can be quite sensitive to prolonged sample storage. If much time is expected to pass between sampling and examination, two air-dried smears on microscope slides should be prepared at the time of sampling (using techniques outlined below) and sent together with the sample. In this way, the original characteristics of the activated sludge will be preserved for conducting Gram and Neisser stains. The slides should be marked with sample identification, date, and G or N (Gram or Neisser), on the side of the slide containing the smear. The same procedure should be followed if samples cannot be analyzed immediately upon receipt.

## 5.2 Staining Procedures

Two primary staining procedures are used in the examination of activated sludge samples—the Gram stain and the Neisser stain. Other procedures with more specialized uses are the "S" test, for sulfur oxidation to detect intracellular sulfur granules; India Ink reverse staining, to detect the presence of large amounts of extracellular polymers; PHB staining, to detect the presence of intracellular storage products such as polyhydroxybutyrate (PHB); and staining with crystal violet, to allow examination of sheaths. The procedures are discussed in the following subsections.

### *5.2.1 Gram stain, modified Hucker method.*

**Preparation:**

**Solution 1:** Prepare the following separately, then combine:

| *A* | | *B* | |
|---|---|---|---|
| Crystal Violet | 2 g | Ammonium oxalate | 0.8 g |
| Ethanol, 95% | 20 ml | Distilled water | 80 ml |

**Solution 2:**

| | |
|---|---|
| Iodine | 1 g |
| Potassium iodide | 2 g |
| Distilled water | 3000 ml |

**Solution 3:**

| | |
|---|---|
| Safranin O (2.5 percent in 95 percent ethanol) | 10 ml |
| Distilled water | 100 ml |

**Procedure:**

1. Prepare thin smears on microscope slides and thoroughly air dry (do not heat fix).
2. Stain 1 min. with Solution 1; rinse 1 sec. with water.
3. Stain 1 min. with Solution 2; rinse well with water.
4. Hold slide at an angle and decolorize with 95 percent ethanol added drop by drop to the smear for 25 sec. *Do not over decolorize.* Blot dry.
5. Stain with Solution 3 for 1 min.; rinse well with water and blot dry.
6. Examine under oil immersion at 1000 × magnification with direct illumination (not phase contrast): blue-violet is positive; red is negative.

*5.2.2 Neisser stain.*

**Preparation:**

**Solution 1:**

Separately prepare and store the following:

| *A* | | *B* | |
|---|---|---|---|
| Methlylene Blue | 0.1 g | Crystal Violet (10 percent w/v in 95% | |
| Ethanol, 95% | 5 ml | ethanol) | 3.3 ml |
| Acetic acid, glacial | 5 ml | Ethanol, 95% | 6.7 ml |
| Distilled water | 100 ml | Distilled water | 100 ml |

Mix 2 parts by volume of A with 1 part by volume of B; prepare fresh monthly.

**Solution 2:**

| | |
|---|---|
| Bismark Brown (1 percent w/v aqueous) | 33.3 ml |
| Distilled water | 66.7 ml |

**Procedure:**

1. Prepare thin smears on microscope slides and thoroughly air dry. *Do not heat fix.*
2. Stain 30 sec. with Solution 1; rinse 1 sec. with water.
3. Stain 1 min. with Solution 2; rinse well with water; blot dry.
4. Examine under oil immersion at 1000× magnification with direct illumination (not phase contrast): blue-violet is positive (either entire cell or intracellular granules); yellow-brown is negative.

### *5.2.3 "S" test (sulfur oxidation)*

**Test A** (modified from Eikelboom 1975)

**Solution:** Sodium sulfide solution ($Na_2S \cdot 9H_2O$) 1.0 g/l (prepare weekly)

**Procedure:**

1. On a microscope slide mix 1 drop of activated sludge sample and 1 drop sodium sulfide solution.
2. Allow to stand open to the air 10–20 min.
3. Place a coverslip on the preparation and gently press to exclude excess solution; remove expelled solution with a tissue.
4. Observe at 1000× using phase contrast. A positive S test is the observation of highly refractive, yellow-colored intracellular granules (sulfur granules) (Figure 22b).

This test, at times, gives variable results. This is due to methodological problems involving the relative concentrations of sulfide and oxygen present (sulfur oxidation is an aerobic process). An alternative sulfur oxidation test, developed by Nielsen (1984), may be used:

**Test B** (modified from Neilsen 1984)

**Solution:** Sodium thiosulfate ($Na_2S_2O_3 \cdot 5H_2O$) 1 g/100 ml

**Procedure:**

1. Allow activated sludge sample to settle, and transfer 20 ml of clear supernatant to a 100 ml Erlenmeyer flask.
2. Add 1–2 ml of activated sludge to the flask.
3. Add 1 ml of thiosulfate solution to the flask (final thiosulfate concentration is 2mM).
4. Shake the flask overnight at room temperature.
5. Observe at 1000× phase contrast, as above.

*5.2.4 India Ink reverse stain.*

**Solution:** India ink (aqueous suspension of carbon black particles)

**Procedure:**

1. Mix one drop of India ink and one drop of activated sludge sample on a microscope slide. Depending on the ink used, the sample volume may need to be reduced.
2. Place on the cover slip and observe at 1000 × using phase contrast.
3. In "normal" activated sludge, the India ink particles penetrate the flocs almost completely, at most leaving a clear center.
4. In activated sludge containing large amounts of exocellular polymeric material, there will be large, clear areas containing a low density of cells.

*5.2.5 PHB (polyhydroxybutyrate) stain.*

**Solution 1:** Sudan Black B (IV), 0.3 percent w/v in 60 percent ethanol

**Solution 2:** Safranin O 0.5 percent w/v aqueous

**Procedure:**

1. Prepare thin smears on a microscope slide and thoroughly air dry.
2. Stain 10 min. with Solution 1; add more stain if the slide starts to dry out.
3. Rinse 1 sec. with water.
4. Stain 10 sec. with Solution 2; rinse well with water; blot dry.
5. Examine under oil immersion at 1000 × magnification with transmitted light: PHB granules will appear as intracellular, blue-black granules while cytoplasm will be pink or clear.

*5.2.6 Crystal Violet sheath stain.*

**Solution:** Crystal Violet, 0.1 percent w/v acqueous solution

**Procedure:**

1. Mix 1 drop activated sludge sample and 1 drop Crystal Violet solution on a microscope slide, cover and examine at 1000 × magnification phase contrast. Cells stain deep violet while the sheaths are clear to pink.

## 5.3 Examination Procedures

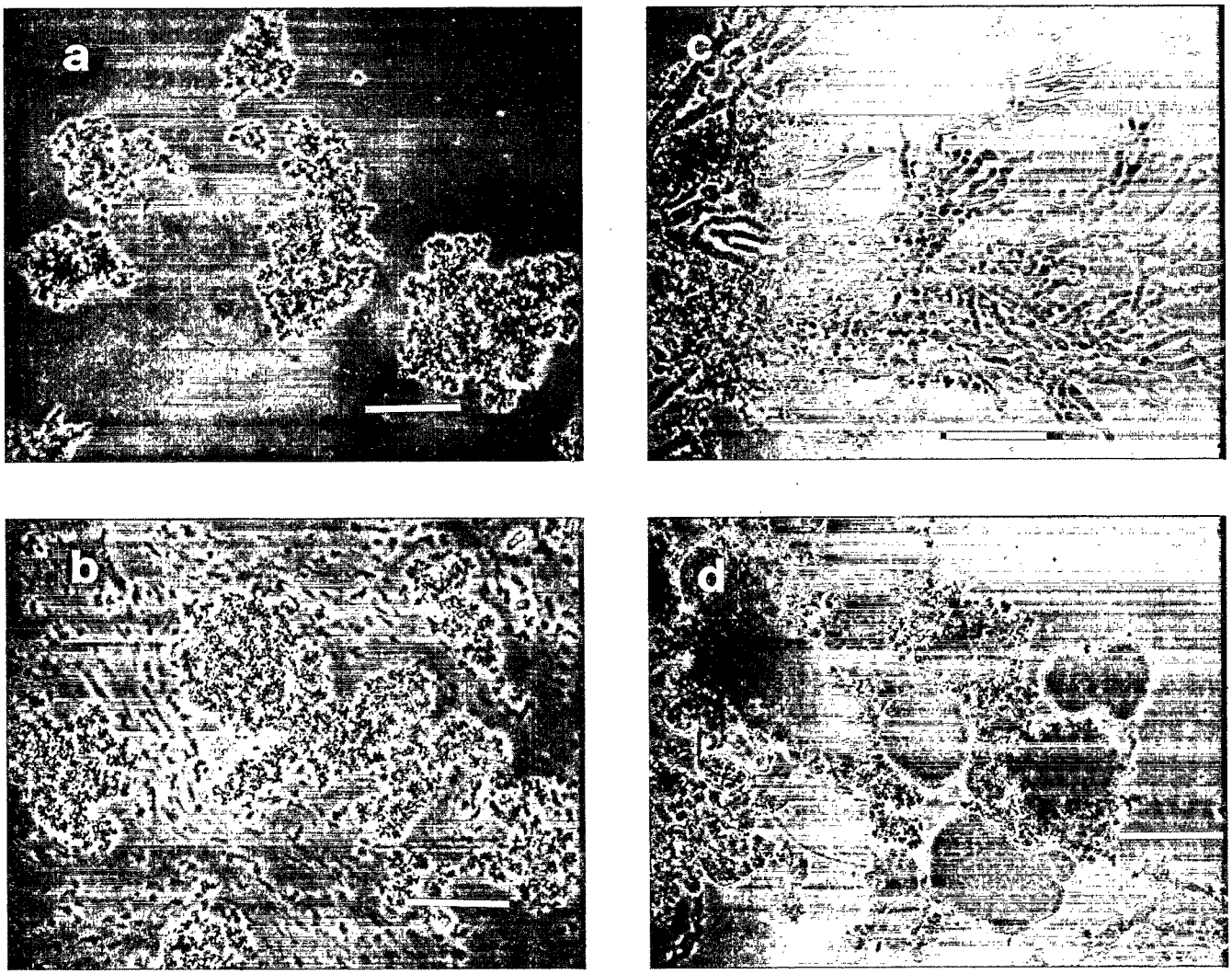
Upon sample receipt, or at least within several hours prior to sample examination, spread 1 drop of the sample evenly over approximately 50 percent of the area of each of two 25 × 77 mm microscope slides. Allow these slides to air dry at room temperature (*do not heat fix*). The slides can be stored and stained later. Mark the slides with a sample identification code and a G or N (Gram or Neisser) on the side on which the smear has been made (frosted end slides are recommended). Perform the Gram and Neisser staining procedures (5.2.1 and 5.2.2).

Withdraw one drop (approximately 0.05 ml) of sample with a loop or a clean, disposable Pasteur pipette and place on a 25 × 75 mm microscope slide. Place a 22 mm No. 1 cover slip on the drop, and press down gently on the cover slip with a blunt object. Remove the liquid that is expelled from the sides of the cover slip with a tissue. This procedure ensures a thin preparation, which is necessary because of the limited depth of focus of the microscope optical system.

Examine the wet mount under phase contrast at 100 × magnification for the following characteristics:

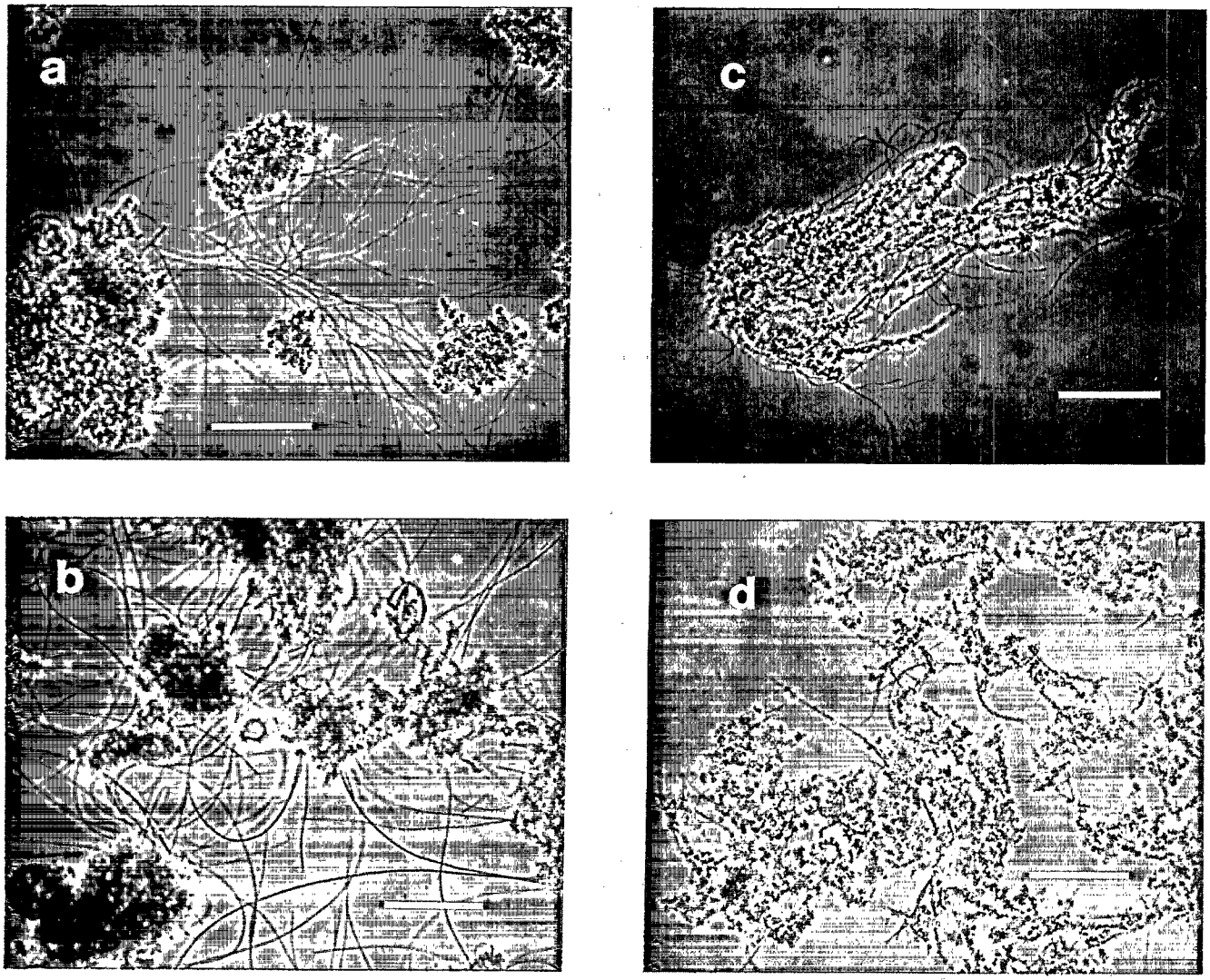
1. The general size and shape of flocs present; measure approximately 10–20 flocs and place them in the following categories:
   a. Size range: small ≤ 150 m diameter
      medium 150–500 μm diameter
      large ≥ 500 μm diameter
   b. Shape: rounded and compact, or irregular and diffuse; note whether texture is firm or weak (Figure 13a and 13b).
2. The presence of protozoa, organic or inorganic particles, and fingered or amorphous zoogleal organisms (Figure 13c and 13d).
3. The presence of free (dispersed) cells in the bulk solution (Figure 13b). Samples containing significant amounts of dispersed cells will appear turbid.
4. The presence and effect of filamentous microorganisms on floc structure, as follows:
   a. None
   b. Bridging—filaments extend from the floc surface into the bulk solution, and bridge between the flocs (Figures 14a and 14b).
   c. Open floc structure—the floc population attaches to and grows around filamentous microorganisms, leading to a large, irregularly shaped floc with substantial internal voids (Figures 14c and 14d).

Figure 13. Floc "texture" in activated sludge (*a* and *b*): *a.* rounded, firm compact; *b.* irregular and diffuse with substantial free cells. Appearance of fingered (*c*) and amorphous (*d*) zoogleal organisms in activated sludge (all 100× phase contrast; bar = 100 μm).




Reference: Jenkins, *et.al.*

Figure 14. Effect of filamentous microorganisms in activated sludge on floc morphology and settleability: *a.* and *b.* inter-floc bridging; *c.* and *d.* diffuse floc structure (all 100× phase contrast; bar = 100 μm).




Reference: Jenkins, et.al.

Table 5. Subjective Scoring of Filament Abundance[a]

| Numerical Value | Abundance | Explanation |
|---|---|---|
| 0 | none | |
| 1 | few | Filaments present but only observed in an occasional floc |
| 2 | some | Filaments commonly observed, but not present in all flocs |
| 3 | common | Filaments observed in all flocs, but at low density (e.g., 1–5 filaments per floc) |
| 4 | very common | Filaments observed in all flocs at medium density (e.g., 5–20 per floc) |
| 5 | abundant | Filaments observed in all flocs at high density (e.g., 20 per floc) |
| 6 | excessive | Filaments present in all flocs—appears more filaments than floc and/or filaments growing in high abundance in bulk solution |

[a]This scale from 0 to 6 represents a 100 to 1,000-fold range of total extended filament length.

5. The abundance of filamentous microorganisms. This can be quantified by several methods. For the purpose of the type of analysis required here, a subjective scoring system is used. Filamentous microorganisms are observed at 100× and subjectively rated for overall abundance on a scale from 0 (none) to 6 (excessive) (Table 5). An abundance rating is determined for the sample as a whole (all filament types together) and for each filamentous microorganism observed. Individual filamentous microorganisms are considered dominant (and probably most responsible for bulking problems) if they are scored "very common" or higher. Organisms are considered secondary (present, but not in sufficient abundance to account for a bulking problem) if they are scored "common" or lower.

   This method is rapid and is suitable for establishing whether a filamentous organism is dominant or secondary. Abundance categories generally are reproducible to within ± one abundance category between observers. Examples of filament abundance categories are shown in Figure 15.

Other methods of microorganism counting are described below.

## 5.4 Counting Procedures

In addition to the subjective scoring method for counting filamentous microorganisms, other more detailed procedures exist for measuring the total extended filament length (TEFL) in activated sludge.

### *5.4.1 Filament measurement technique of Sezgin et al. (1978).*

1. Transfer 2 ml of a well-mixed activated sludge sample of known suspended solids concentration using a wide-mouth pipette (0.8 mm diameter tip) to 1 liter of distilled water in a 1.5-liter beaker and stir at 95 rpm on a jar test apparatus ($G = 85\ sec^{-1}$) for 1 min.

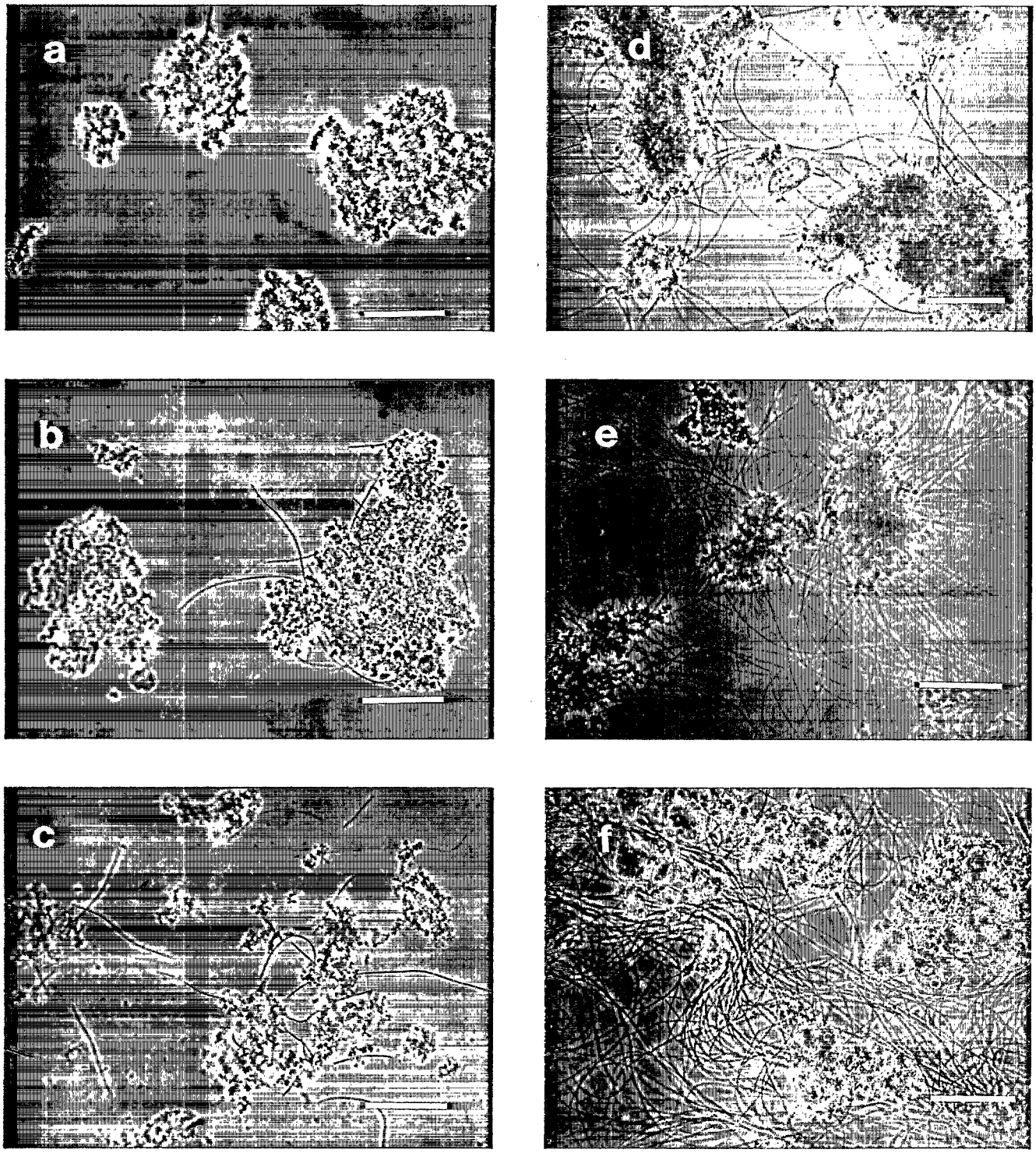
2. Using the same pipette, transfer 1.0 ml diluted sample to a microscopic counting chamber calibrated to contain 1.0 ml and cover with a glass cover slip.

3. Using a binocular microscope at 100× magnification with an ocular micrometer scale, count the number of filaments present in the whole chamber or a known portion of it and place these in the following size classifications: 0 to 10 μm, 10 to 25 μm, 25 to 50 μm, 50 to 100 μm, 100 to 200 μm, 200 to 400 μm, 400 to 800 μm, and greater than 800 μm. Measure filaments of length greater than 800 μm individually.

4. Express results as the total μm of filament length per g or ml of MLSS:

   total extended filament length (TEFL) μm/gMLSS = total filament length, μm, in the 1.0 ml diluted sample × the dilution factor (× 500 in the above example) ÷ MLSS concentration, g/l

Figure 15. Filament abundance categories using subjective scoring system: *a.* few; *b.* some; *c.* common; *d.* very common; *e.* abundant; and *f.* excessive (all 100× phase contrast; bar = 100 μm).




Reference: Jenkins, *et.al.*

total extended filament length (TEFL) $\mu$m/ml MLSS = total filament length, m, in the 1.0 ml diluted sample × the dilution factor (× 500 in the above example).

### *5.4.2 Diluted SVI procedure of Stobbe (1984).*

Several researchers have found close correlations between the number of filamentous microorganisms in sludge and a parameter known as Diluted Sludge Volume Index (DSVI). The DSVI can be used to predict sludge settling properties and is well correlated to TEFL.

1. Set up several 1-liter graduated cylinders (the number will depend on prior knowledge of the settleability of the sludge).
2. Using well-clarified secondary effluent, prepare a series of two-fold dilutions of the activated sludge (i.e., no dilution, 1:1 dilution; 1:3 dilution).
3. Stir the graduate cylinders individually for 30–60 sec. using a plunger to resuspend and uniformly distribute the sludge solids.
4. Allow the activated sludge to settle for 30 min. under quiescent conditions.
5. Observe the settled sludge volume ($SV_{30}$) in the graduated cylinder where the settled volume is less than and closest to 200 ml ($SV_{30} < 200$ ml).
6. Calculate the diluted SVI using:

$$\text{DSVI (ml/g)} = \frac{SV_{30}\ \text{(ml/l)} \times 2^n}{SS\ \text{(g/l)}}$$

where n is the number of two-fold dilutions required to obtain a settled sludge volume ($SV_{30}$) less than 200 ml and SS is the suspended solids concentration of the *undiluted* activated sludge.

### *5.4.3 Simplified filament counting technique used by the San Jose/Santa Clara (California) Water Pollution Control Plant (SJ/SCWPCP).*

This method does not directly measure TEFL. Rather, it counts the number of times filaments intersect a single line on the microscope eyepiece, for several fields of view across a wet mount preparation of the activated sludge sample.

1. Transfer 50 $\mu$l mixed liquor sample to a glass slide.
2. Cover completely with a 22 × 30 mm cover slip.
3. Using 100× total magnification and starting at the edge of the cover slip, observe consecutive fields across the entire 30 mm length of the cover slip. At SJ/SCWPCP this is 17 fields.
4. The eyepiece is fitted with a single hairline. Count the number of times that any filamentous microorganism intersects the hairline.
5. Total the number of intersections for all fields examined. This is the Filament Count. If a "unit count" is desired, the Filament Count must be multiplied by the number of fields in the 22 mm width of the slide. At San Jose this is 12 fields. Thus

$$\text{Filament Count}/\mu\text{l} = \frac{\left(\dfrac{\text{(filament intersections in)}}{\text{field counted}}\right)}{50\ \mu\text{l}} \times 12$$

SJ/SCWPCP has correlated filament count with SVI. However, filament count cannot provide an early warning for problems caused by increased SVI, because the two are temporally correlated.

## Chapter 6

## 6.0 Appendix 2—Microscopic Identification of Filamentous Microorganisms

The other important part of microscopic study of activated sludge samples is identification of the type(s) of filamentous microorganisms present in the sludge.

## 6.1 Observation of Microorganism Characteristics

Scan the sample at 100× using phase contrast to ascertain the number of different types of filamentous microorganisms present. Carefully characterize each filamentous microorganism present by looking at several filaments of each type and expressing the results as an "average." Use count sheets such as the ones presented in Table 6 to record and summarize observations.

This task can be simplified by accepting only a limited number of descriptions, and learning to recognize special features that provide clues to the filamentous microorganism type. These features are:

1. *Branching*—present or absent; if present, whether true or false branching.

   True branching is cell branching where there is a contiguous cytoplasm between branched trichomes (Figure 16a, 16b, and 16c). In activated sludge the only branched trichome-forming microorganisms are fungi and *Nocardia* spp., and *Nostocoida limicola* (observed incidentally). False branching occurs when there is no continuous cytoplasm between trichomes; two trichomes have merely stuck together and grown outward (Figure 16d). In activated sludge, false branching usually is only observed for *Sphaerotilus natans*, but also has been reported incidentally for type 1701.

2. *Motility*—none, or, if present, describe.

   Only a few filamentous microorganisms in activated sludge are motile. *Beggiatoa* spp., *Flexibacter* spp., and some blue-green bacteria (*Cyanophyceae*) are motile by gliding; *Thiothrix* spp. and type 021N may display limited "twitching" or swaying motions.

3. *Filament shape*—straight, smoothly-curved, bent, irregularly-shaped "chain of cells," coiled, or mycelial (Figure 17).

4. *Color*—transparent, medium, dark (Figure 18a, 18b, and 18c).

5. *Location*—extending from floc surface, found mostly within the floc, or free in the liquid between flocs (Figure 18d, 18e and 18f).

6. *Attached growth of epiphytic unicellular bacteria*—present or absent; if present, whether substantial growth or incidental (Figure 19).

7. *Sheath*—present or absent.

   The presence of a sheath is one of the most difficult characteristics to establish. A true sheath is a clear structure (hence, hard to observe) exterior to the cell wall. Sheaths can be seen best in unstained preparations when they are empty of the cells, or when some of the cells are missing. In the latter case the outline of the sheath can be seen continuing along either side of the empty space (Figure 20). Several features of filamentous microorganisms can be confused with sheaths. A yellowish "halo" observed around filaments under phase contrast observation is not a sheath, but an artifact of phase contrast illumination. Short, empty spaces in a trichome or at the trichome apex should be used to indicate the presence of a sheath. The cell wall of some filamentous microorganisms may remain after cell lysis; however, this can be distinguished from a sheath because some evidence of pre-existing cross-walls usually remains. This is commonly observed for type 021N.

   Presence of substantial attached growth generally indicates the presence of a sheath. Staining wet mounts with Crystal Violet (Section 5.2.6) may aid in sheath detection (Figure 20f).

8. *Cross-walls (cell septa)*—present, absent.

   This feature can be variable for some filamentous microorganisms, and detection is dependent on the quality and adjustment of the microscope. It

**Table 6. Suggested Format for Filamentous Microorganism Identification Worksheet**

No. _____ Sample _____

SAMPLE DATE ____ / ____ / ____ OBSERVATION DATE ____ / ____ / ____

FILAMENT ABUNDANCE

| ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
|---|---|---|---|---|---|---|
| 0 None | 1 Few | 2 Some | 3 Common | 4 Very Common | 5 Abundant | 6 Excessive |

FILAMENT EFFECT ON FLOC STRUCTURE: Little or None Bridging Open Floc Structure

MORPHOLOGY OF FLOC: ☐ Firm ☐ Round, Compact

☐ Weak ☐ Irregular, Diffuse

FLOC DIAMETER ( m)

| 150 | 150–500 | 500 |
|---|---|---|
| | | |

FEATURES:

Free cells in suspension _____

Zoogloea's _____

Inorganic/Organic Particles _____

FILAMENTOUS MICROORGANISM SUMMARY:

| | Rank | Abundance | | Rank | Abundance |
|---|---|---|---|---|---|
| *Nocardia* sp. | | | *M. parvicella* | | |
| type 1701 | | | type 0581 | | |
| *S. natans* | | | type 0092 | | |
| type 021N | | | type 0803 | | |
| *Thiothrix* sp. | | | type 1851 | | |
| type 0041 | | | type 0961 | | |
| *H. hydrossis* | | | other | | |
| *N. limicola* | | | other | | |

x = Dominant 0 = Secondary

REMARKS: _____

**Reference: Jenkins, *et al.*, 1984.**

Table 6 (continued).

No. _____ Sample _____

COMMENTS:

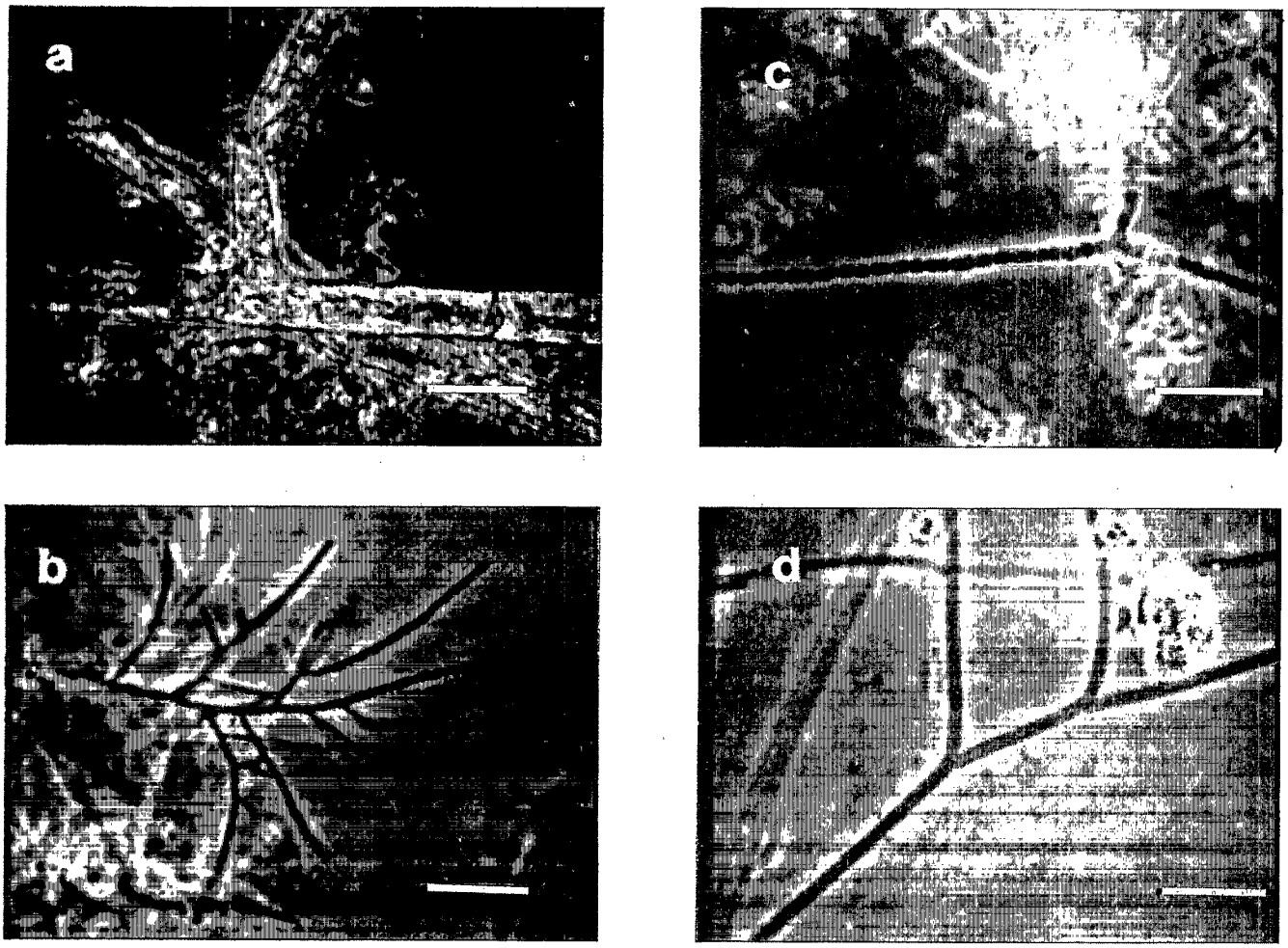
OBSERVATION OF:
Protozoa
Metazoa:

*WET MOUNT OBSERVATION, 1000X, PHASE CONTRAST:*

| FILAMENT # | A | B | C | D | E |
|---|---|---|---|---|---|
| BRANCHING | | | | | |
| MOTILITY | | | | | |
| FILAMENT SHAPE | | | | | |
| COLOR | | | | | |
| LOCATION | | | | | |
| ATTACHED UNICELLS | | | | | |
| SHEATH | | | | | |
| CROSSWALLS | | | | | |
| FILAMENT DIAMETER | | | | | |
| LENGTH | | | | | |
| CELL SHAPE | | | | | |
| SIZE | | | | | |
| SULFUR DEPOSITS | | | | | |
| OTHER GRANULES | | | | | |
| COMMONNESS | | | | | |
| RANK | | | | | |
| *STAINS, 1000X* | | | | | |
| GRAM | | | | | |
| NEISSER | | | | | |
| I.D. | | | | | |

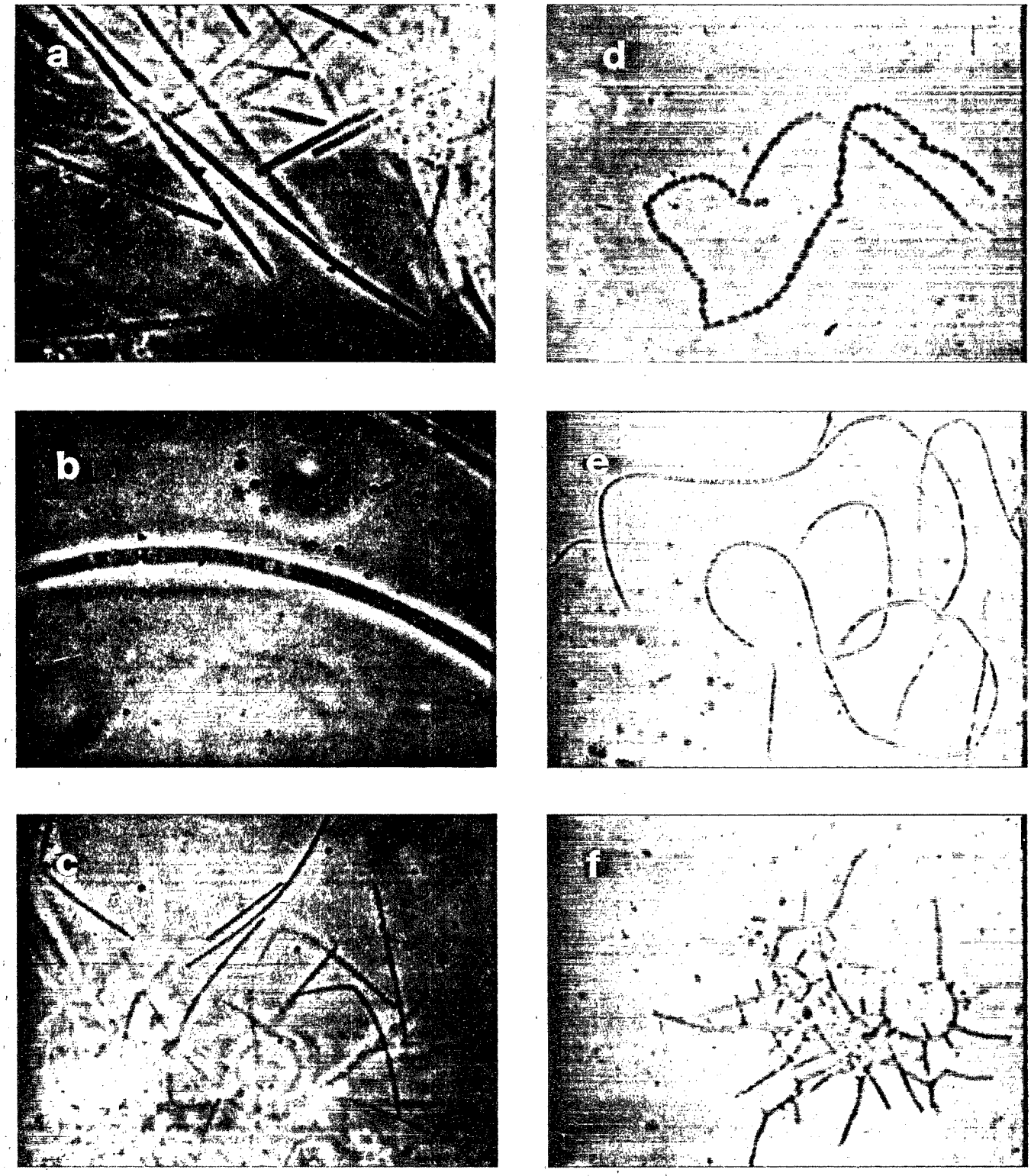
Reference: Jenkins, *et al.*, 1984.

Figure 16. Trichome branching observed for filamentous microorganisms in activated sludge: *a., b.* and *c.* true branching (fungus, *Nocardia* sp., and *Nostocoida limicola* II respectively); *d.* false-branching (*Sphaerotilus natans*) (all 1000× phase contrast; bar = 10 μm).



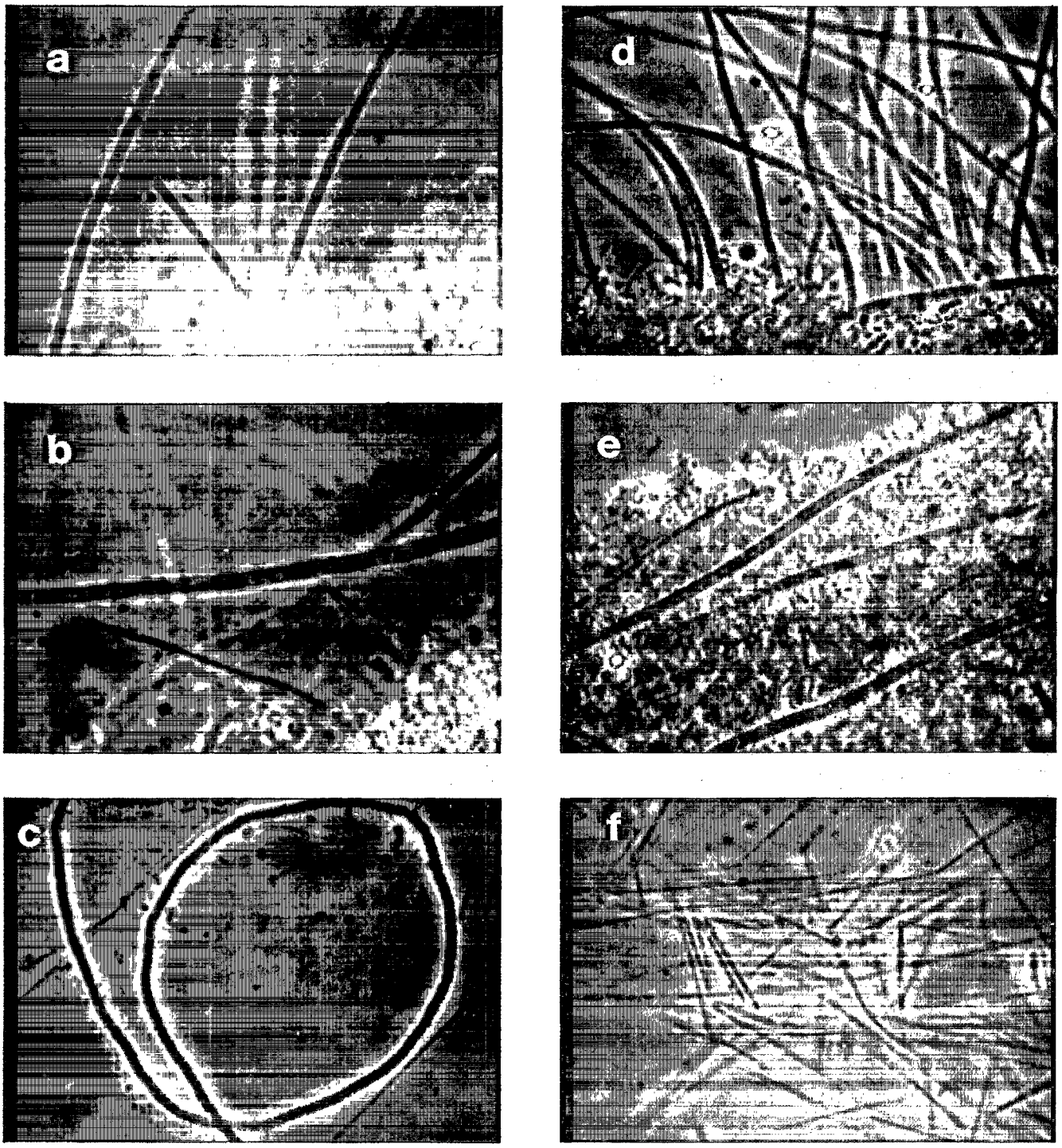

Reference: Jenkins, *et.al.*

Figure 17. Examples of filament shapes: *a.* straight; *b.* smoothly-curved; *c.* bent; *d.* irregularly-shaped "chain of cells"; *e.* coiled; and *f.* mycelial (all 1000× phase contrast).



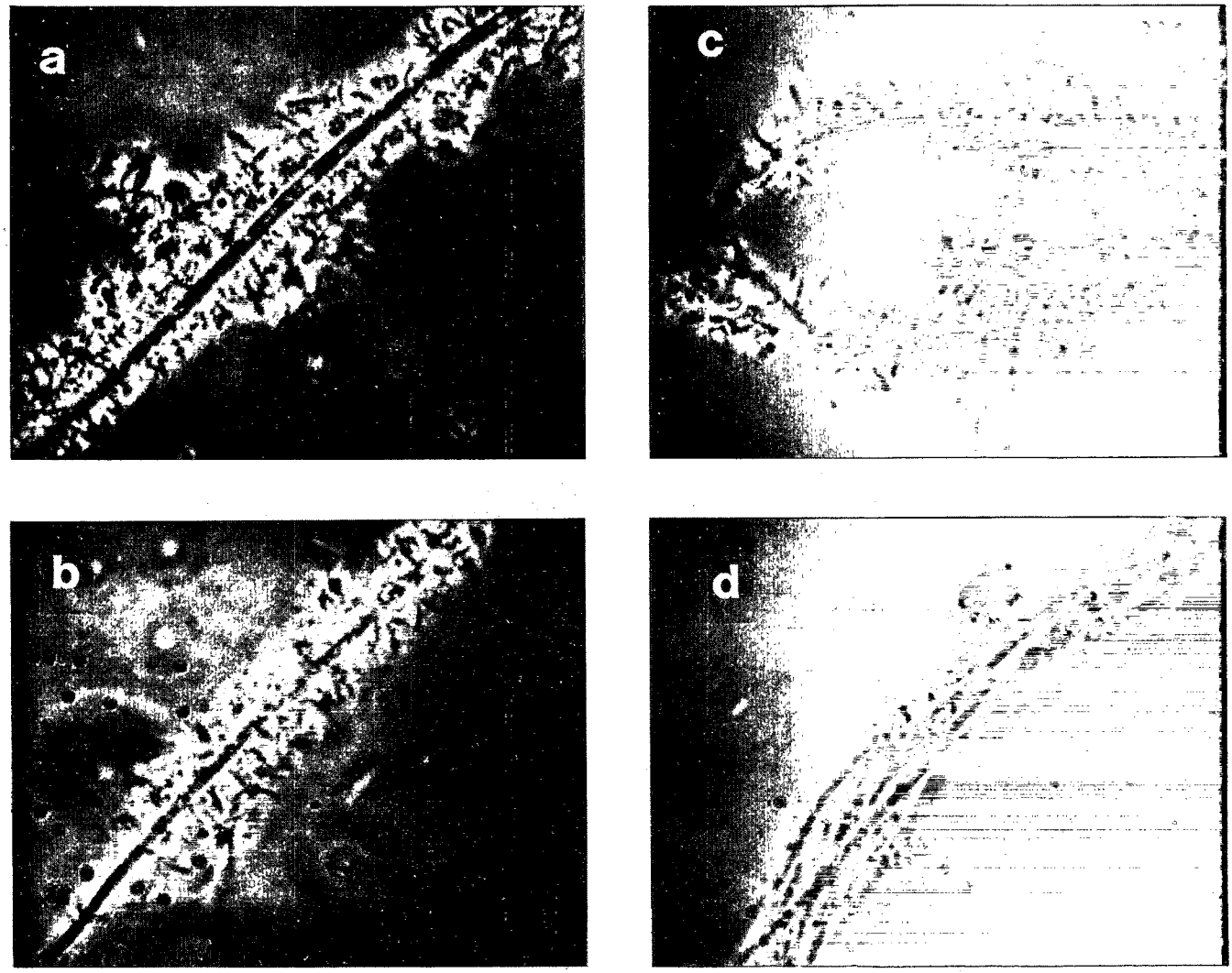

Reference: Jenkins, *et.al.*

Figure 18. "Color" of filamentous microorganisms (*a, b* and *c*): *a.* transparent; *b.* medium; and *c.* dark. Location of filaments in activated sludge (*d, e* and *f*): *d.* extending from floc surface; *e.* most within the floc; and *f.* free (all 1000× phase contrast).



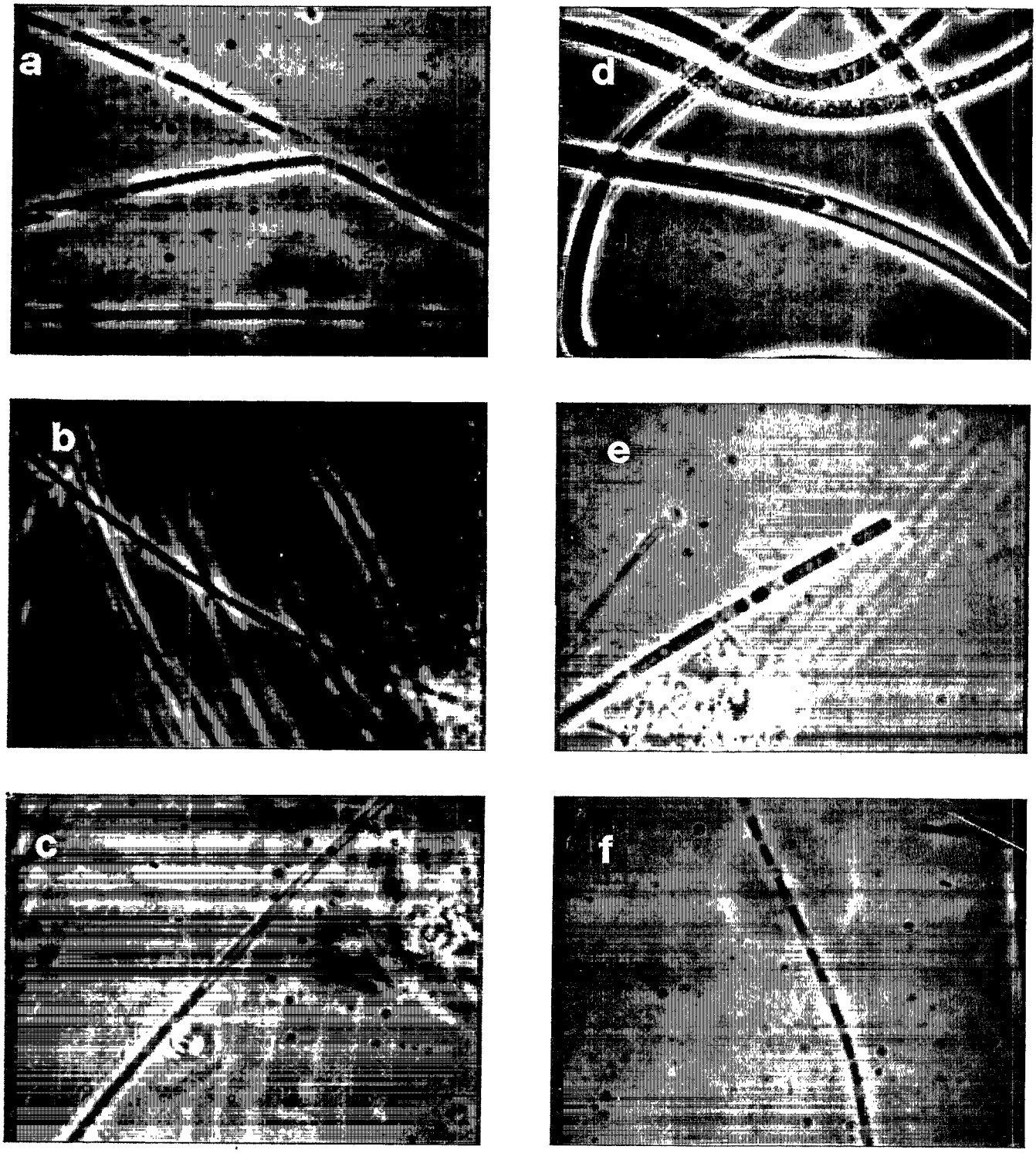

Reference: Jenkins, *et al.*, 1984.

Figure 19. Attached growth of epiphytic bacteria on filamentous microorganisms: *a.*, *b.* and *c.* substantial (types, 0041, 0675 and 1701 respectively); *d.* incidental (type 1851) (all 1000× contrast).



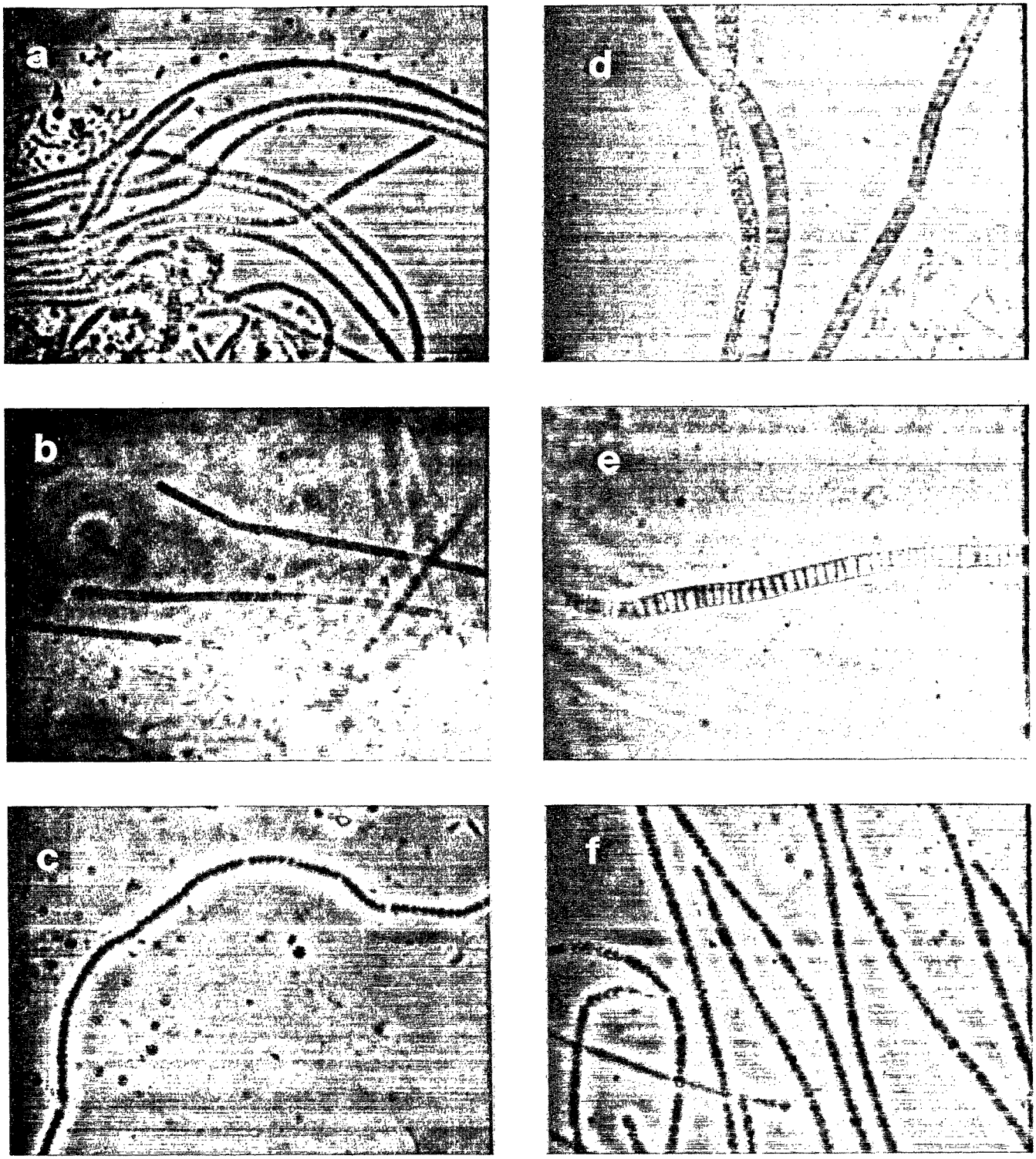

Reference: Jenkins, *et al.*, 1984.

Figure 20. Appearance of sheaths: *a. Sphaerotilus natans; b.* type 1701; *c. Thiothrix* II; *d. Thiothrix* I; *e.* type 0041; and *f.* type 1701, stained with crystal violet (all 1000× phase contrast).




Reference: Jenkins, *et al.*, 1984.

Figure 21. Cell shapes observed for filamentous microorganisms in activated sludge: *a.* square; *b.* rectangular; *c.* oval; *d.* barrel; *e.* discoid; and *f.* round-ended rods (all 1000× phase contrast).




Reference: Jenkins, *et al.*, 1984.

is important to determine whether a true trichome is present (Figure 17b) or whether the filament is made up of a chain of cells (Figure 17d).

9. *Filament diameter*—Both the average diameter and its range in $\mu$m should be measured; it is important to note whether the diameter is greater than 1 $\mu$m or less than 1 $\mu$m.

10. *Filament length*—range in $\mu$m.

11. *Cell shape*—square, rectangular, oval, barrel, discoid, round-ended rods (Figure 21).

    It is important to note whether there are indentations at cell septa (Figure 21c, 21d, 21e, and 21f) or whether trichome walls are straight at the cell junctions (Figure 21a and 21b).

12. *Size*—average length and width of cells in $\mu$m.

13. *Sulfur deposits*—present or absent in situ and present or absent after the S test (Section 5.2.3) (Figures 22a and 22b).

    Under phase contrast observation, sulfur granules appear as bright yellow-colored cell inclusions, either in the shape of spheres observed for *Thiothrix* spp., *Beggiatoa* spp., and type 021N (Figure 22c, 22d, and 22e); or in the shape of squares for type 0914 (Figure 22f). Type 0914 does not respond to the S test.

14. *Other granules*—present or absent. Commonly observed granules are polyphosphate (Neisser positive granules), and PHB (confirmed by PHB staining) (Section 5.2.5).

15. *Staining reactions*—Each filamentous microorganism present is separately evaluated for Gram staining and Neisser staining reaction by observing the stained smears at 900–1000× using transmitted light (not phase contrast). The position and length of filamentous microorganisms in the wet mount and the presence or absence of attached growth should be carefully noted so that the same filament types can be examined in the stained smears. Care is required in this observation because some filamentous microorganisms change size upon drying and staining (e.g., type 0092 appears much wider when Neisser stained than in wet mounts).

    The Gram stain requires much practice. Reagents should be reasonably fresh (3–6 months) and, if possible, should be tested on fresh cultures of known Gram reaction. The decolorization step should be controlled precisely to avoid overdecolorization. Also, large flocs do not decolorize fully, so the Gram reactions inside large flocs should be ignored.

    Score the Gram reaction as positive, negative, or variable. Most filamentous microorganisms observed in activated sludge are Gram negative (Figure 23a and 23b). *Nostocoida limicola* and types 0041 and 0675 most often are Gram positive but can be Gram variable or Gram negative (Figure 23c). Type 1851 stains weakly Gram positive, and generally is observed as a chain of Gram positive "beads" (Figure 23d). *Thiothrix* I, *Beggiatoa* spp., type 021N, and type 0914 generally stain Gram negative, but may stain Gram positive when they contain substantial intracellular sulfur deposits. *Microthrix parvicella* and *Nocardia* spp. are generally strongly Gram positive (Figure 23e and 23f).

    Neisser staining is a straightforward technique. Score as negative, positive (entire trichome is stained), or negative with Neisser-positive granules (Figure 24).

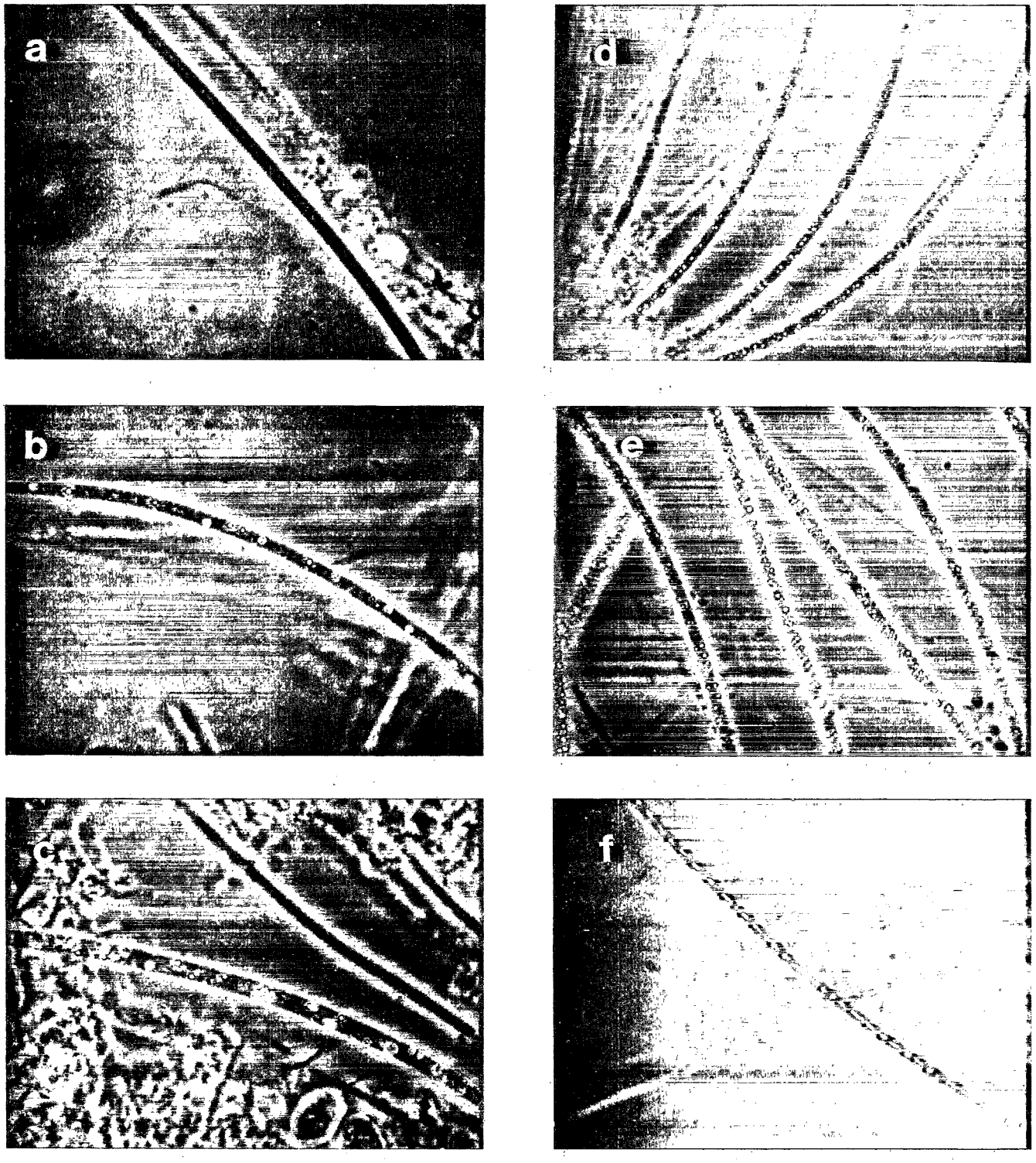
    Type 0092 (light purple—Figure 24c) and *N. limicola* (dark purple—Figure 24d) stain entirely Neisser-positive. *M. parvicella* and *Nocardia* spp. stain Neisser-negative but generally contain Neisser-positive intracellular granules (Figure 24e and 24f). *Beggiatoa* spp., *Thiothrix* spp., and types 0041, 0675, 021N, 0914 and 1863 may contain Neisser-positive granules (infrequently). In addition, *H. hydrossis* and types 0675 and 0041 may have a Neisser-positive trichome "covering" (Figure 24b) when present in activated sludge that is nutrient-deficient.

16. *Additional observations*—Two filamentous microorganisms, *Thiothrix* spp. and type 021N (uncommonly) may display rosettes and gonidia. A rosette develops when trichomes radiate outward from a common origin (Figure 25). Gonidia are ovalor rod-shaped cells present at the trichome apex that are distinctively different in appearance from vegetative cells (Figures 25, 26, and 27).

    The following observations may indicate a nutrient deficiency (e.g., N and/or P) in activated sludge systems:

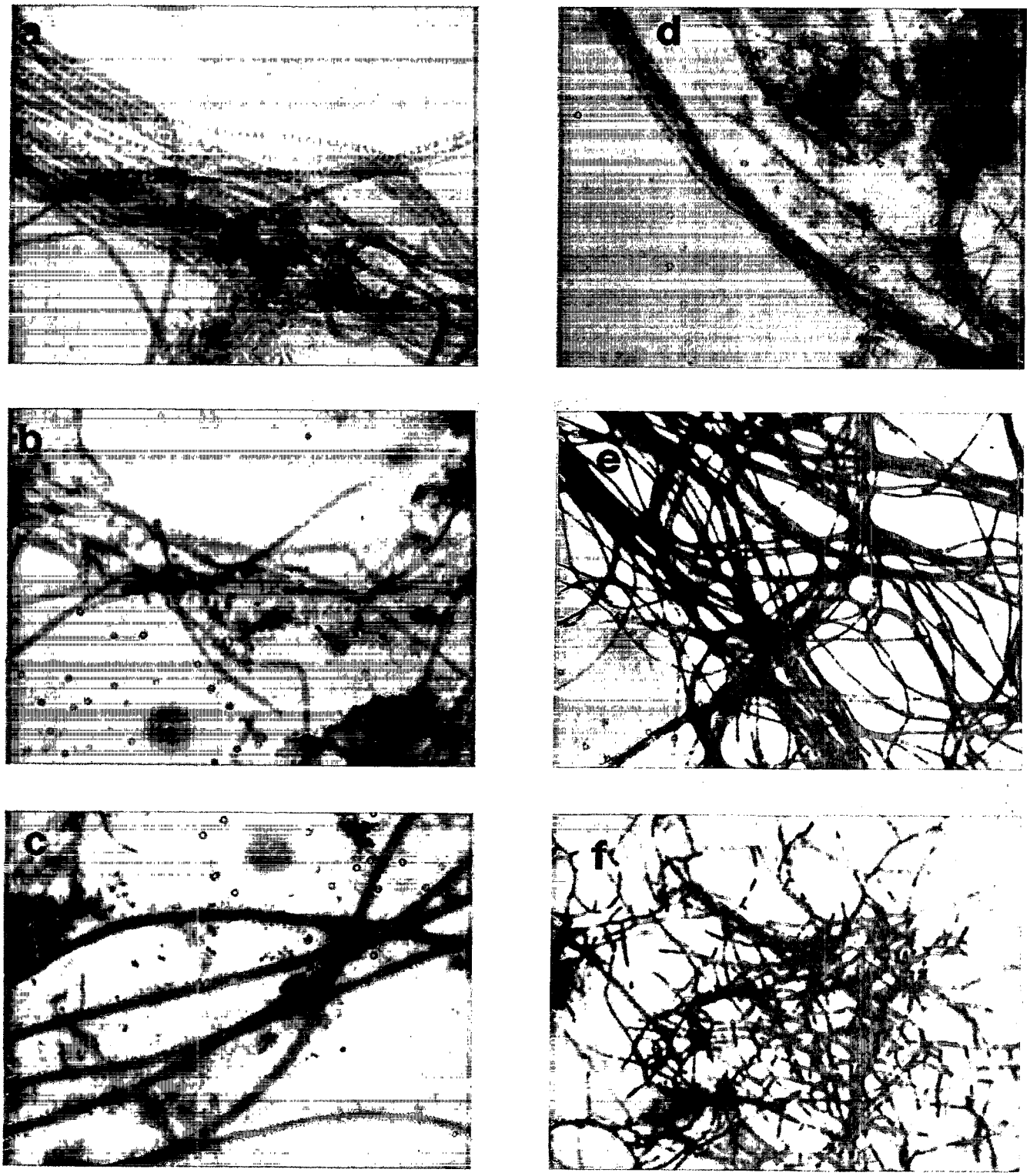
    - Large amounts of intracellular PHB granules;
    - Unusual Neisser-staining reactions of some filamentous microorganisms (see above); and
    - Large amounts of extracellular material in the flocs. This is detected by conducting the India ink negative staining procedure (Section 5.2.4). When observed at 100× phase contrast, the Indian ink particles normally can be seen to penetrate deeply into the flocs, leaving only a clear center; when there are large,

Figure 22. Deposition of intracellular sulfur granules during the S test (*a* and *b*): before (*a*) and after (*b*) adding sodium sulfide. Appearance of intracellular sulfur granules in filamentous microorganisms (*c, d, e* and *f*): *c. Thiothrix* I; *d. Thiothrix* II; *e.* type 021N; and *f.* type 0914 (all 1000× phase contrast).



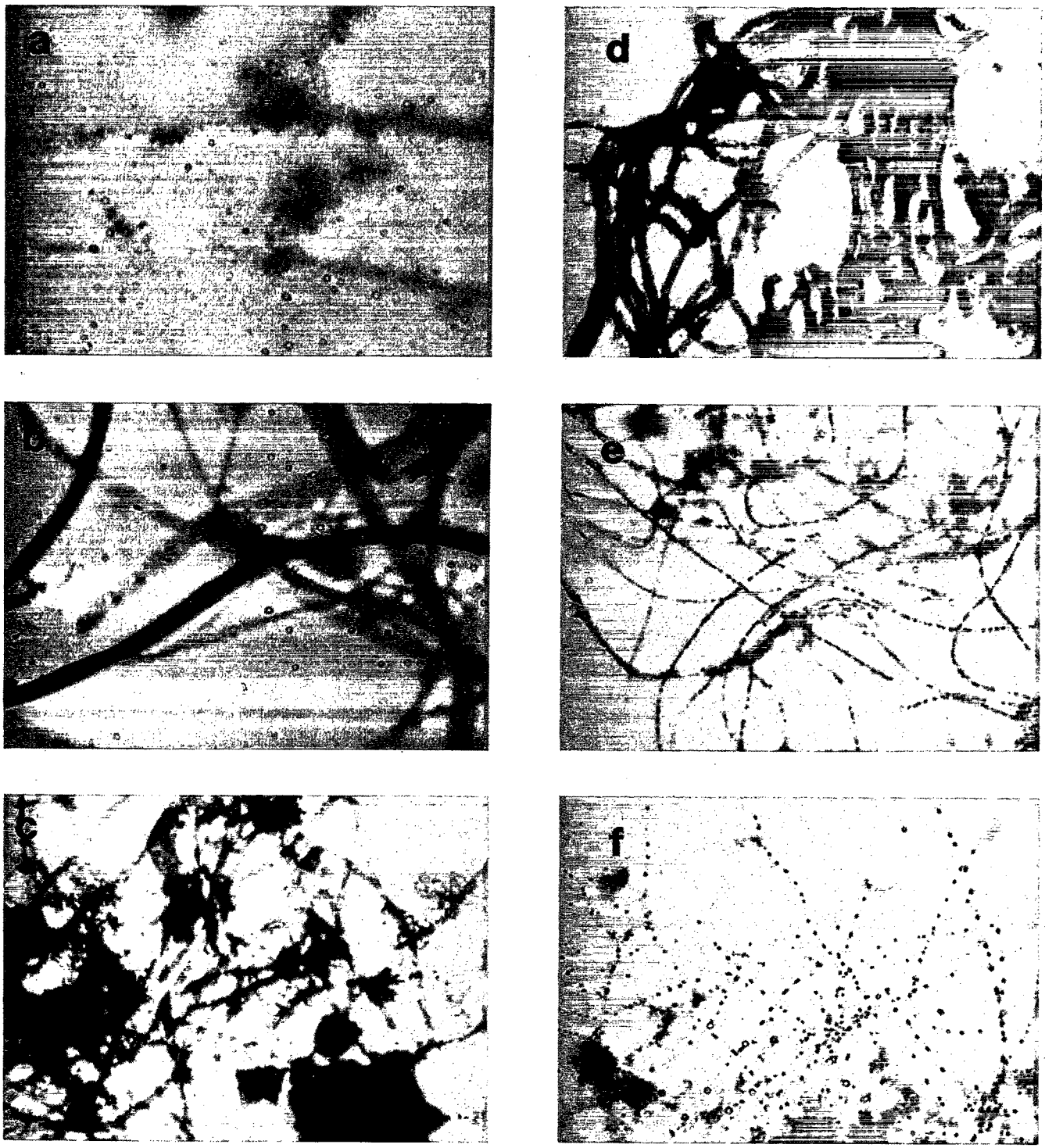

Reference: Jenkins, *et al.*, 1984.

**Figure 23.** Gram stain reaction of filamentous microorganisms: *a.* and *b.* Gram negative (types 021N and 0092 respectively); *c.* Gram variable (type 0041); *d.* weakly Gram positive (type 1851); and *e.* and *f.* Gram positive (*Microthrix parvicella* and *Nocardia* spp. respectively) (all 1000× transmitted light).



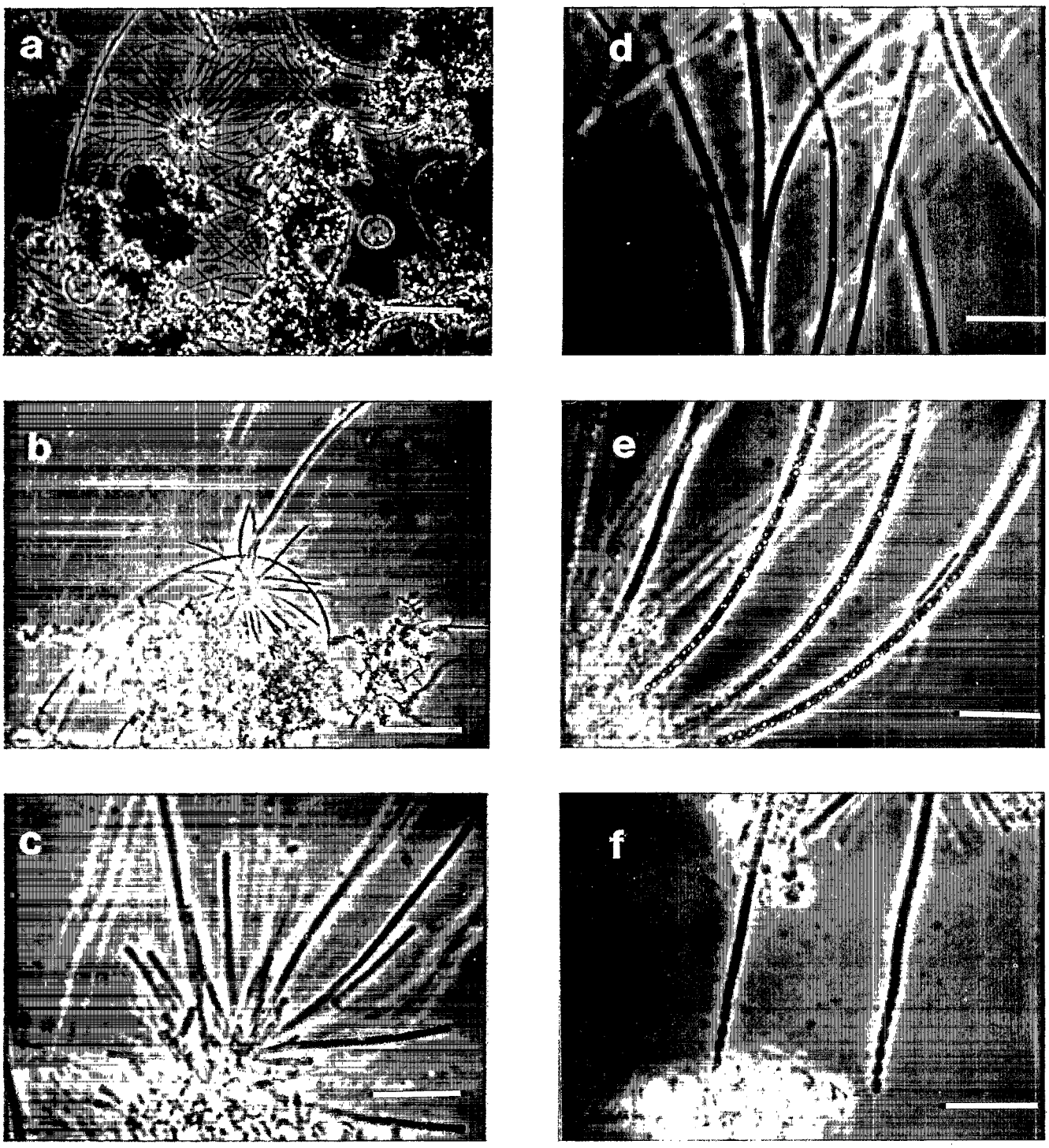

**Reference:** Jenkins, *et al.*, 1984.

Figure 24. Neisser staining reaction of filamentous microorganisms: *a.* negative; *b.* Neisser positive trichome covering observed atypically for type 0041; *c.* and *d.* Neisser positive (type 0092 and *Nostocoida limicola* II respectively); and *e.* and *f.* Neisser positive granules (*Microthrix parvicella* and *Nocardia* spp. respectively) (all 1000× transmitted light).



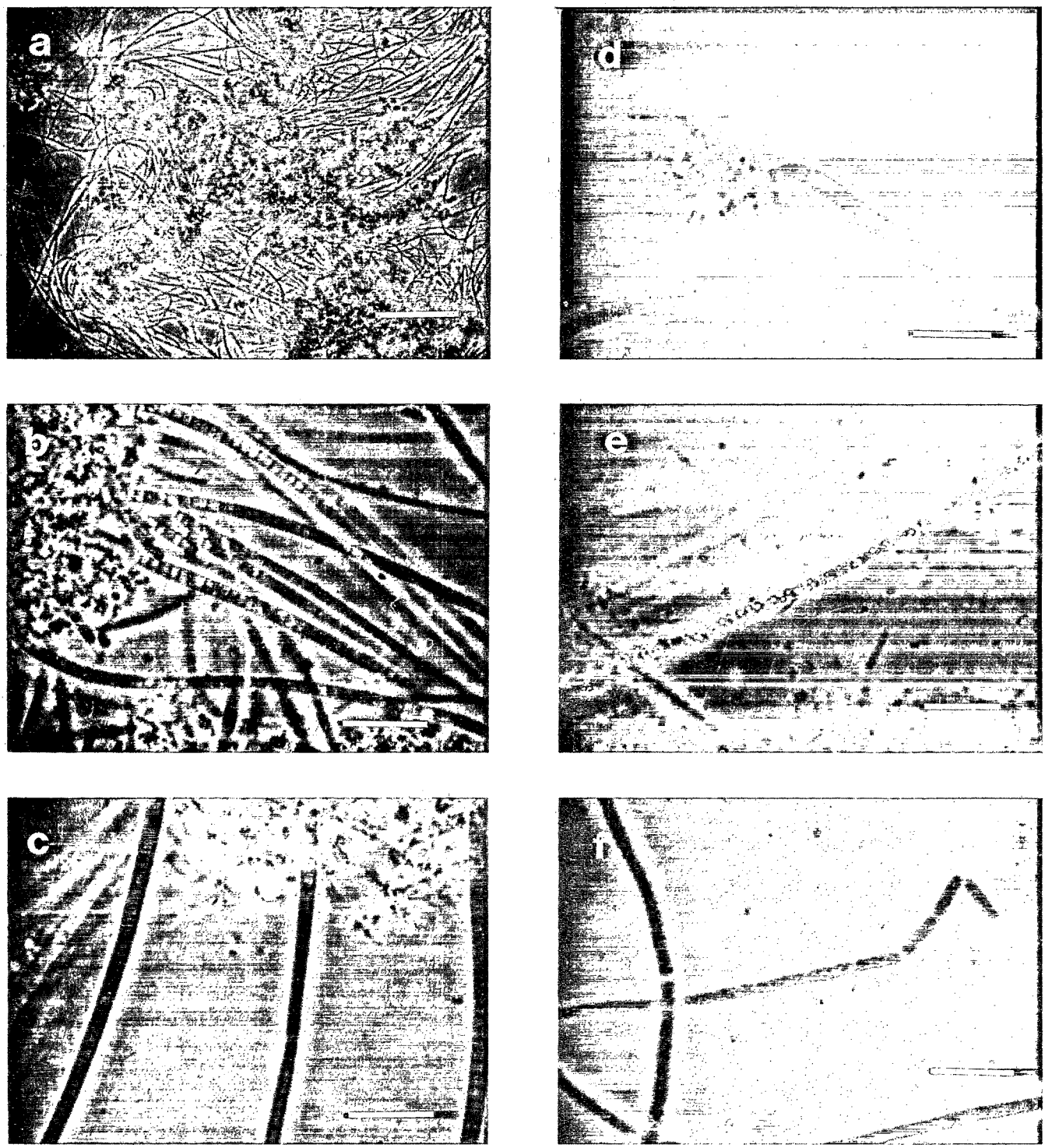

Reference: Jenkins, *et al.*, 1984.

Figure 25. *Thiothrix* II (*a* and *b* 100× phase contrast; bar = 100 μm; *c–f* 1000× phase contrast; bar = 10 μm).



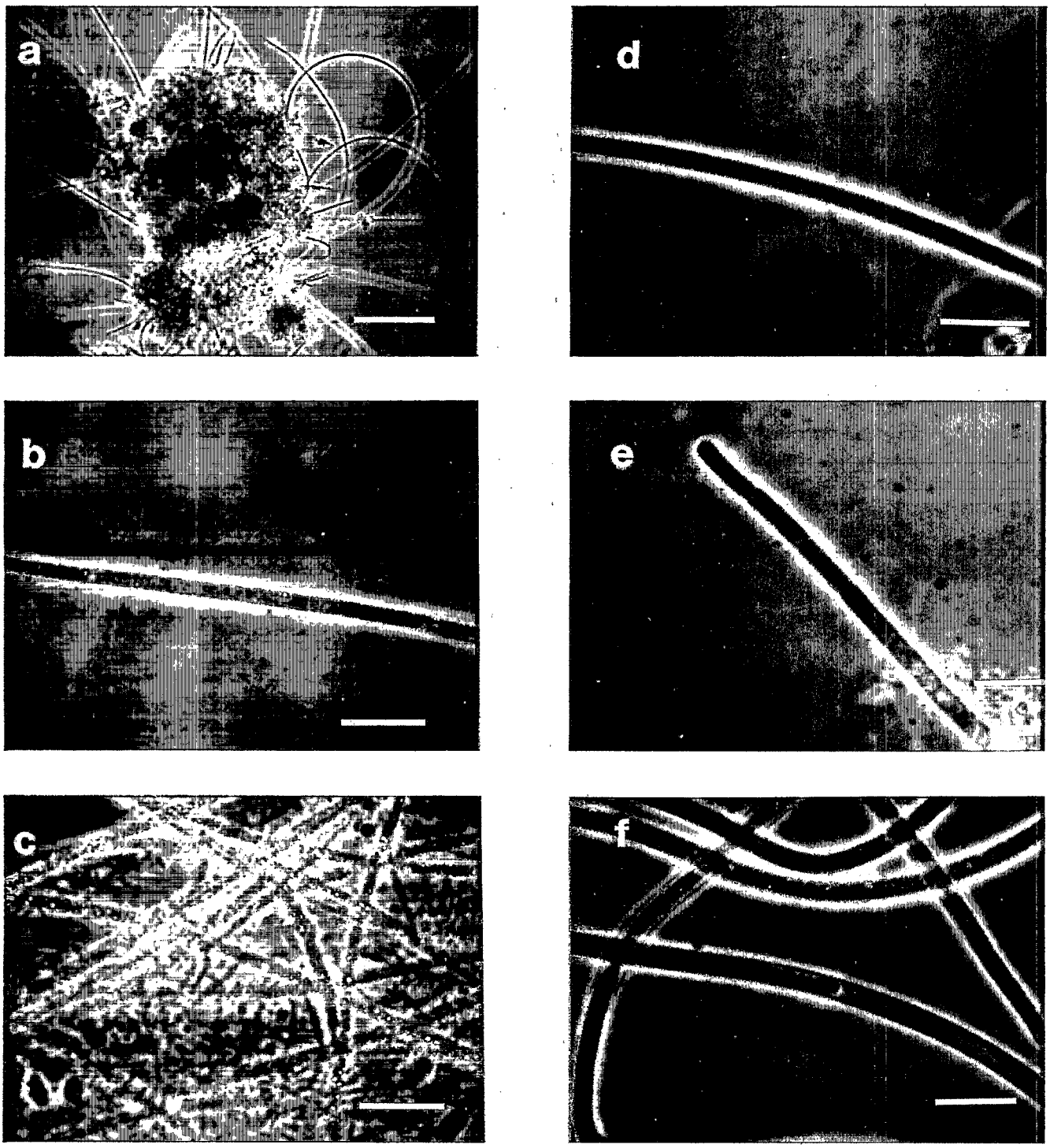

Reference: Jenkins, *et al.*, 1984.

Figure 26. Type 021N (*a* 100× phase contrast; bar = 100 μm; *b–f* 1000× phase contrast; bar = 10 μm).




Reference: Jenkins, *et al.*, 1984.

Figure 27. *Thiothrix* I (*a* 100× phase contrast; bar = 100 μm; *b–f* 1000× phase contrast; bar = 10 μm).




Reference: Jenkins, *et al.*, 1984.

clear areas with low cell density, the presence of large amounts of extracellular material (probably polysaccharide) is indicated.

Additional clues pointing to the presence of large amounts of this highly water-retentive extracellular material are: the activated sludge settles and compacts poorly even though filamentous microorganism abundance is low; the activated sludge is slippery or slimy to the touch and appears viscous when poured.

## 6.2 Identification of Microorganisms

Observed and recorded filament characteristics are used to characterize the filamentous microorganisms by genus or by a numbered type using the dichotomous key shown in Figure 28. This key lists the 22 filamentous bacteria most commonly observed in activated sludge. To simplify this key, several filamentous microorganisms having readily identifiable specific characteristics are not listed, but are described later. These include: fungi, *Cyanophyceae, Flexibacter* spp., and *Bacillus* spp. In addition, some filamentous microorganisms observed only occasionally in activated sludge are not included in the key. These include filament types 1702, 1852, and 0211.

This dichotomous key is a modification of the filamentous microorganism identification key given by Eikelboom and van Buijsen (1981), with changes to:

- Deemphasize the need for observation of cell septa (crosswalls), which can depend on the quality and adjustment of the microscope used; and
- Include some filamentous microorganisms in the key twice where an important characteristic is variable, e.g., Gram stain reaction for *N. limicola* II and the observation of intracellular sulfur granules for types 0914 and 021N.

The use of this key is not without risk because some filamentous microorganism characteristics vary, and the key cannot always address all of these variables. The filament type arrived at using the key should be carefully checked against the typical microorganisms characteristics listed in Table 7, and presented in the short descriptions and the photographs of each organism that follow. If characteristics given in Table 7, or in the photographs, do not correspond to the filament type arrived at using the key, careful re-examination of characteristics used in the key is in order. For example, type 0041 generally gives a weak Gram positive or a Gram variable reaction, and as such, is keyed correctly from Figure 28. However, if strongly Gram positive, it would be keyed as *N. limicola* II. Reference to Table 7 shows *N. limicola* II to be coiled and to possess no attached growth—type 0041 is straight or smoothly-curved and most often has substantial attached growth.

Occasionally, a filamentous microorganism is observed that is not represented by a type or genus designation. Such a filamentous microorganism should be reported as "not identified"; do not try to "force-fit" the microorganism into existing filament types.

## 6.3 Descriptions of Types of Filamentous Microorganisms

These short descriptions of each filamentous microorganism commonly observed in activated sludge are based on information given by Eikelboom and van Buijsen (1981) as modified by experience with filamentous microorganism characteristics in activated sludges from the USA and South Africa. The following filament types usually are observed in domestic wastewater treatment plants at conventional organic loading rates:

| | | |
|---|---|---|
| *S. natans* | *Beggiatoa* spp. | *H. hydrossis* |
| type 1701 | *Nocardia* spp. | type 1863 |
| type 021N | *N. limicola* II | |
| *Thiothrix* I and II | | |

Filament types observed in industrial or domestic wastewater treatment plants operated at low organic loading rates are:

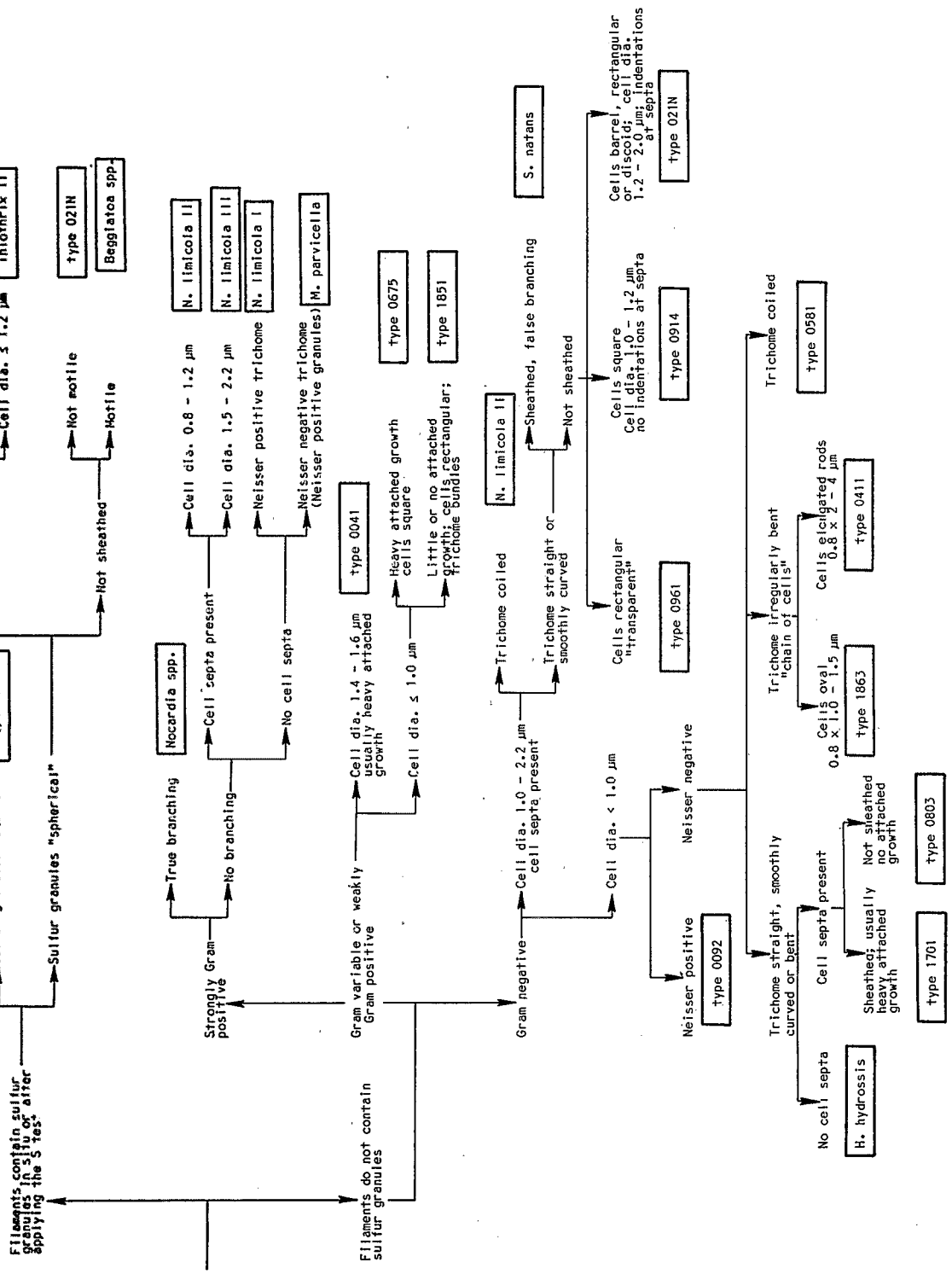
| | | |
|---|---|---|
| type 0041 | *Beggiatoa* spp. | *M. parvicella* |
| type 0675 | type 1851 | *Nocardia* spp. |
| type 021N | type 0803 | *N. limicola* I, II, III |
| *Thiothrix* I and II | type 0092 | *H. hydrossis* |
| type 0914 | type 0961 | type 0581 |

Filament types that are observed infrequently include:

| | | |
|---|---|---|
| fungi | *Flexibacter* spp. | type 0211 |
| *Cyanophyceae* | type 1702 | type 0411 |
| *Bacillus* spp. | type 1852 | |

1. *Sphaerotilus natans.* (Figure 29a, b, and c; see also Figures 16d and 20a.) Relatively long (100–1000 $\mu$m) straight or smoothly-curved filaments composed of round-ended, rod-shaped cells (1.0–1.8 × 1.5–3.0 $\mu$m) contained in a clear, tightly-fitting sheath. Cell septa are clear with indentations at septa. Filaments radiate outward from the floc surface into the bulk solution. False branching frequently is observed, giving a "tree-branch"-like appearance. Gram negative, Neisser negative, no sulfur granules: PHB frequently observed. Cell shape can be rectangular when the cells are tightly packed within the sheath. An exocellular slime coating may occur at nutrient limitation. Attached growth uncommon, but may occur when not growing.

**Figure 28. Dichotomous key for filamentous microorganism "identification" in activated sludge.**




**Reference: Jenkins, *et al.*, 1984.**

**Table 7. Summary of Typical Morphological and Staining Characteristics of Filamentous Microorganisms Commonly Observed in Activated Sludge**

| BRIGHT FIELD OBSERVATION | | | | PHASE CONTRAST OBSERVATION 1000X | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | NEISSER STAIN | | SULFUR GRANULES | | | | | | | | | | | |
| FILAMENT TYPE | GRAM STAIN | trichome | granules | *in situ* | S test | OTHER CELL INCLUSIONS | TRICHOME DIAMETER | TRICHOME LENGTH | TRICHOME SHAPE | TRICHOME LOCATION | CELL SEPTA CLEARLY OBSERVED | INDENTATIONS AT CELL SEPTA | SHEATH | ATTACHED GROWTH | CELL SHAPE AND SIZE | NOTES |
| S. natans | – | – | – | – | – | PHB | 1.0 - 1.4 | 500 | St | E | + | + | + | – | round-ended rods 1.4 × 2.0 | False branching |
| type 1701 | – | – | – | – | – | PHB | 0.6 - 0.8 | 20 - 80 | St,B | I,E | + | + | + | + + | round-ended rods 0.8 × 1.2 | cell septa hard to discern |
| type 0041 | +,V | – | –,+ | – | – | – | 1.4 - 1.6 | 100 - 500 | St | I,E | + | – | + | + +,– | squares 1.4 × 1.5 - 2.0 | Neisser positive reaction occurs |
| type 0675 | +,V | – | –,+ | – | – | – | 0.8 - 1.0 | 50 - 150 | St | I | + | – | + | + +,– | squares 1.0 × 1.0 | Neisser positive reaction occurs |
| type 021N | – | – | –,+ | –,+ | + | PHB | 1.0 - 2.0 | 50 - 500 | St,SC | E | + | + | – | – | barrels, rectangles, discoid 1-2 × 1.5 - 2.0 | rosettes, gonidia |
| Thiothrix I | –,+ | – | –,+ | +,– | + | PHB | 1.4 - 2.5 | 100 - 500 | St,SC | E | + | – | + | – | rectangles 2.0 × 3-5 | rosettes, gonidia |
| Thiothrix II | – | – | –,+ | +,– | + | PHB | 0.8 - 1.4 | 50 - 200 | St,SC | E | + | – | + | – | rectangles 1.0 × 1.5 | rosettes, gonidia |
| type 0914 | –,+ | – | –,+ | –,+ | – | PHB | 1.0 | 50 - 200 | St | E,F | + | – | – | – | squares 1.0 × 1.0 | sulfur granules "square" |
| Beggiatoa spp. | –,+ | – | –,+ | +,– | + | PHB | 1.2 - 3.0 | 100 - 500 | St | F | –,+ | – | – | – | rectangles 2.0 × 6.0 | motile: flexing and gliding |
| type 1851 | + weak | | – | – | – | – | 0.8 | 100 - 300 | St,B | E | +,– | – | + | –,+ | rectangles 0.8 × 1.5 | trichome bundles |
| type 0803 | – | – | – | – | – | – | 0.8 | 50 - 150 | St | E,F | + | – | – | – | rectangles 0.8 × 1.5 | |
| type 0092 | – | + | – | – | – | + | 0.8 - 1.0 | 20 - 60 | St,B | I | +,– | – | – | – | rectangles 0.8 × 1.5 | |
| type 0961 | – | – | – | – | – | – | 0.8-1.2 | 40 - 80 | St | E | + | – | – | – | rectangles 1.0 × 2.0 | "transparent" |
| M. parvicella | + | – | + | – | – | PHB | 0.8 | 100 - 400 | C | I | – | – | – | – | – | large "patches" |
| Nocardia spp. | + | – | + | – | – | PHB | 1.0 | 10 - 20 | I | I | +,– | – | – | – | variable 1.0 × 1 - 2 | true branching |
| N. Limicola I | + | + | – | – | – | – | 0.8 | 100 | C | I,E | – | – | – | – | – | |
| N. Limicola II | –,+ | +,– | – | – | – | PHB | 1.2 - 1.4 | 100 - 200 | C | I,E | + | + | – | – | discs, ovals 1.2 × 1.0 | Incidental branching Gram and Neisser variable |
| N. Limicola III | + | + | – | – | – | PHB | 2.0 | 200 - 300 | C | I,E | + | + | – | – | disc, ovals 2.0 × 1.5 | |
| H. hydrossis | – | – | – | – | – | – | 0.5 | 20 - 100 | St,B | E,F | – | – | + | –,+ | – | "rigidly straight" |
| type 0581 | – | – | – | – | – | – | 0.5 - 0.8 | 100 - 200 | C | I | – | – | – | – | – | |
| type 1863 | – | – | –,+ | – | – | – | 0.8 | 20 - 50 | B,I | E,F | + | + | – | – | oval rods 0.8 × 1-1.5 | "chain of cells" |
| type 0411 | – | – | – | – | – | – | 0.8 | 50 - 150 | B,I | E | + | + | – | – | elongated rods 0.8 × 2-4 | "chain of cells" |

*notation:* + = positive; – = negative; V = variable; single symbol invariant; +,– or –,+, variable, the first being most observed.
Trichome shape: St = straight; B = bent; SC = smoothly curved; C = coiled; I = Irregularly-shaped.
Trichome location: E = extends from floc surface; I = found mostly within the floc; F = Free in liquid between the flocs.

**Reference: Jenkins, *et al.*, 1984.**

2. *Type 1701.* (Figure 29c, d, and e; see also Figures 19c and 20f.) Relatively short (10–100 μm), curved or bent filaments composed of round-ended, rod-shaped cells (0.7–1.0 × 1.0–2.0 μm) contained in a clear, tightly fitting sheath. Cell septa are clear with indentations at septa. Filaments found predominantly intertwined within the floc interior with only short filaments extending into the bulk solution. No branching. Gram negative, Neisser negative, no sulfur granules; PHB frequently observed. Attached growth of epiphytic bacteria is almost always observed, making observation of individual cells difficult.

3. *Type 0041.* (Figure 30a, b, and c; see also Figures 19a, 19e, and 23c.) Straight, smoothly-curved or bent filaments, 100–500 μm in length, composed of square-shaped cells (1.2–1.6 × 1.5–2.5 μm) contained in a clear, tightly fitting sheath. In domestic waste systems, it is most often observed inside the floc and covered with heavy, attached growth. In industrial waste systems, it may occur extending from the floc surface or free in the bulk solution, and may not have any attached growth. Gram positive or Gram variable, tending to Gram positive when found within the floc and Gram negative when extending into the bulk solution. Neisser negative; Neisser-positive granules are observed infrequently; and a Neisser-positive (light purple color) slime coating may be observed in some industrial waste systems (Figure 24). Intracellular granules are rarely observed. No sulfur granules. The sheath is difficult to detect and is observed when cells are missing, particularly at the trichome apex (Figure 30).

4. *Type 0675.* (Figure 30*d, e,* and *f.*) Very similar to type 0041, only smaller in trichome length (50–150 μm) and cell dimension (0.7–1.0 μm). Covered with heavy, attached growth in domestic waste activated sludge; may lack attached growth in some industrial waste activated sludge. A sheath is present. Gram positive to Gram variable, Neisser negative. Neisser-positive granules occur; no sulfur granules.

5. *Type 021N.* (Figure 26a–f; see also Figures 21d and e, 31e, and 23a.) Filaments typically are 1.0–2.0 μm in width and 100–500 μm in length and taper from a thicker basal region, often exhibiting an inconspicuous hold-fast, to a thinner apical region, often terminating in loosely-attached gonidia. Trichomes are straight, smoothly-curved or sometimes slightly coiled and are found extending from the floc surface. Cell shape ranges from ovoid to rectangular or barrel-shaped with clear cell septa and indentations at septa. Gram negative and Neisser negative; may contain Neisser-positive granules. Cells may stain slightly Gram positive when they contain sulfur granules. Spherical intracellular sulfur granules are observed in situ infrequently; response to the S test generally is positive. Rosettes are observed infrequently. No attached growth. No sheath is present; however, a heavy cell wall (still showing cross septa) often remains after cell lysis.

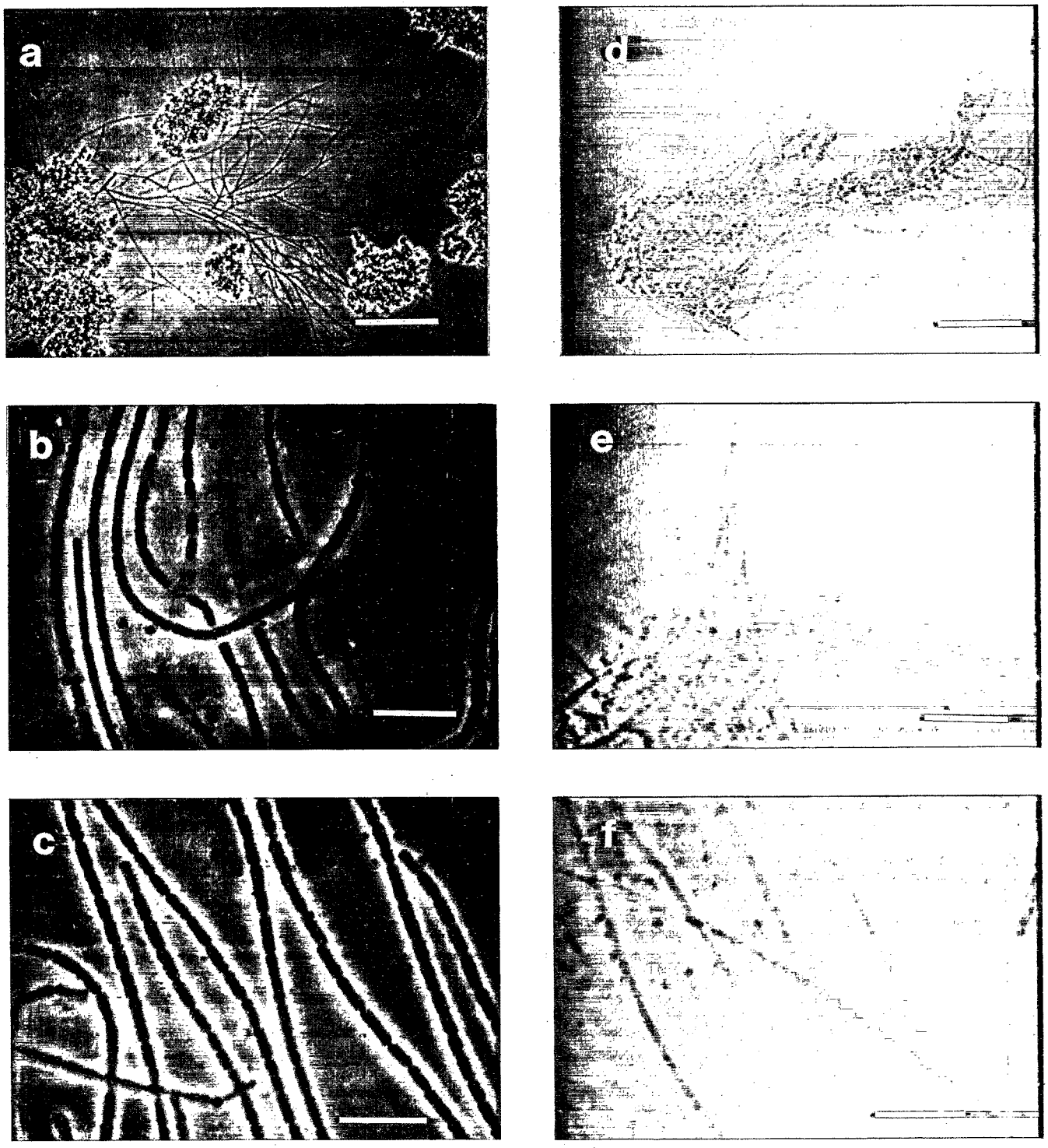
6. *Thiothrix I.* (Figure 27a–f; see also Figure 22c.) Straight or smoothly curved trichomes, 1.4–2.5 μm in width and 100–500 μm in length, found extending from the floc surface. Cells are rectangular (1.4–2.5 × 3–5 μm) with clear cell septa without indentations at septa. No attached growth. A sheath is present. Gram negative and Neisser negative; however, a Gram-positive reaction may occur when sulfur granules are present. Neisser-positive granules may occur. Cells frequently contain sulfur granules in situ and this organism responds strongly to the S test. Apical gonidia commonly are observed, and an inconspicuous hold-fast is present.

7. *Thiothrix II.* (Figure 25a–f; see also Figures 20c and 22d.) Straight or smoothly-curved filaments, 0.8–1.4 μm in width and 50–200 μm in length, found extending from the floc surface. No attached growth. Cell septa without indentations are present. Cells are rectangular (0.8–1.4 × 1–2 μm). Gram negative and Neisser negative, with Neisser-positive granules sometimes present. Cells frequently contain spherical sulfur granules in situ and this organism responds to the S test. Apical gonidia and rosettes are commonly observed. Trichomes may taper somewhat from base to tip. A sheath is present, but difficult to detect.

8. *Type 0914.* (Figure 31a, b, and c.) Straight or smoothly-curved filaments, 0.7–1.0 μm in width and 50–200 μm in length, found extending from the floc surface or more commonly free in the bulk solution. May have incidental attached growth. Cells are square-shaped (1.0 × 1.0 μm) without constrictions at septa. No sheath is present. Gram negative and Neisser negative, but may stain Gram positive when substantial sulfur granules are present. Neisser positive granules may occur. May contain intracellular sulfur granules, which appear square rather than spherical as observed for other filamentous microorganisms. Does not respond to the S test.

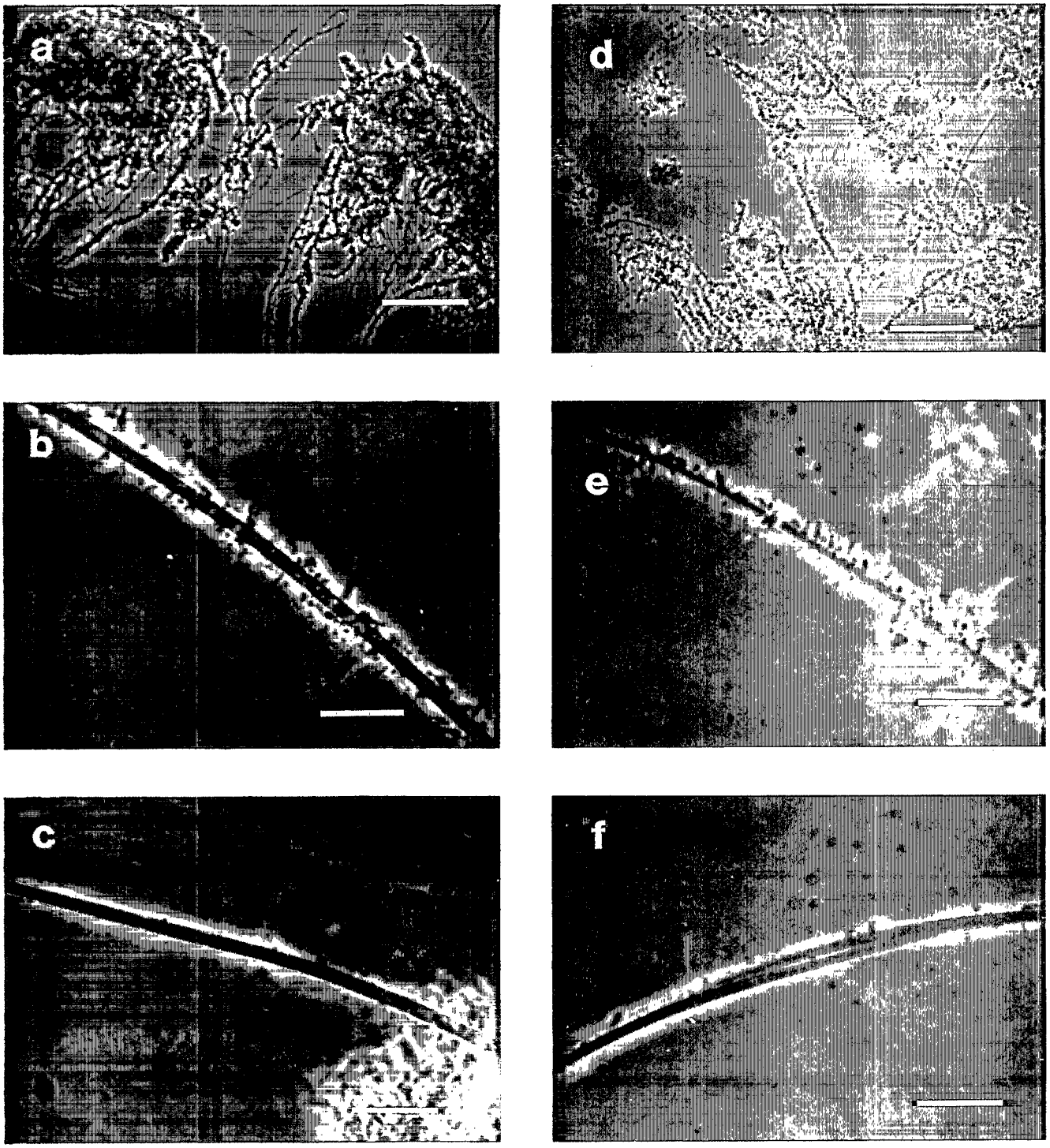
9. *Beggiatoa* spp. (Figure 32a and b.) Large, straight filaments, 1.0–3.0 μm in width and 100–500 μm in length, found free in the bulk solution and

Figure 29. *Sphaerotilus natans* (*a, b* and *c*) and type 1701 (*d, e* and *f*) (*a* and *d* 100× phase constrast; bar = 100 m; *b, c, e* and *f* 1000× phase contrast, bar = 10 $\mu$m).



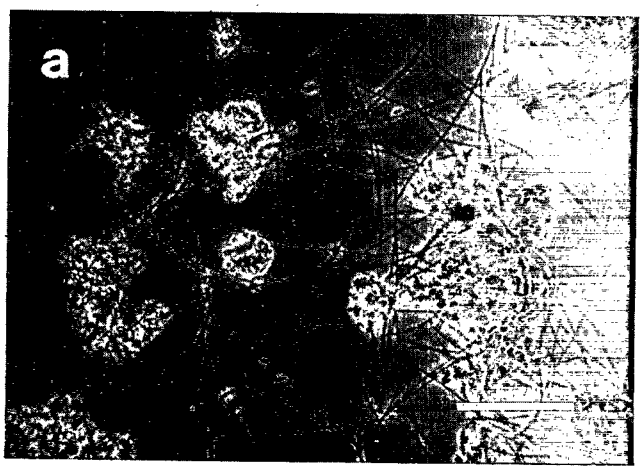

Reference: Jenkins, *et al.*, 1984.

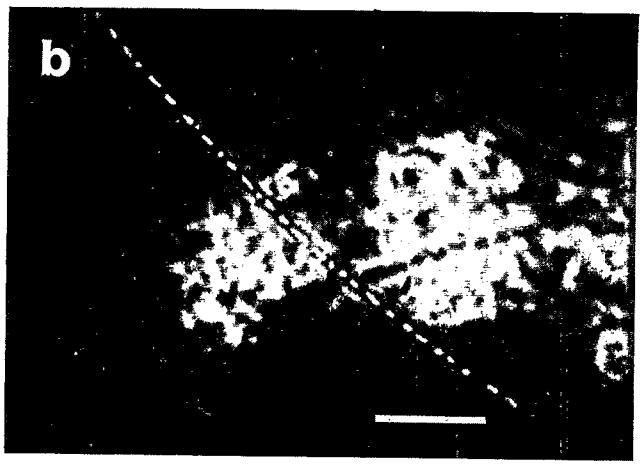
Figure 30. Type 0041 (*a, b* and *c*) and type 0675 (*d, e* and *f*) (*a* and *d* 100× phase contrast; bar = 100 μm; *b, c, e* and *f* 1000× phase contrast; bar = 10 μm).

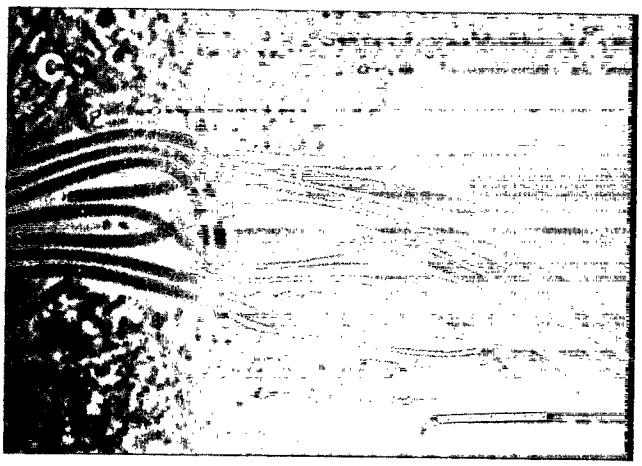



Reference: Jenkins, *et al.*, 1984.

Figure 31. Type 0914 (*a* 100× phase contrast; bar = 100 μm; *b* and *c* 1000× phase contrast; bar = 10 μm) (note sulfur granules in *b*).




Reference: Jenkins, *et al.*, 1984.

actively motile by gliding and flexing. Generally contains substantial spherical sulfur granules; cell septa are not visible when sulfur is present. Without sulfur, cells are rectangular (1–3 × 4–8 μm). No attached growth. No sheath. Gram negative and Neisser negative; however, cells may stain Gram positive when substantial sulfur is present. Neisser-positive granules may occur.

10. *Type 1851.* (Figure 32c and d; see also Figures 19d and 23d.) Straight or bent trichomes, 0.8 μm in width and 100–300 μm in length, observed extending from the floc surface, or more commonly in bundles of intertwined filaments. A sheath is present, but hard to observe. Cells are rectangular (0.8 × 1.5–2.5 μm) without indentations at septa, or with indentations that are difficult to observe. Attached growth occurs, which is distinctly perpendicular to the trichome surface. Weakly Gram positive (a Gram-positive "beaded" effect) and Neisser negative. No sulfur granules.

11. *Type 0803.* (Figure 32e.) Straight or smoothly-curved filaments of uniform diameter (0.8 μm) and 50–150 μm in length, found extending from the floc surface or at times free in the bulk solution (industrial waste activated sludges). No sheath and no attached growth. Cells are rectangular (0.8 × 1.5–2.0 μm) without constrictions at septa. Gram negative and Neisser negative. No sulfur granules.

12. *Type 0092.* (Figure 32f; see also Figures 23b and 24c.) Straight, irregularly-curved or bent filaments, 0.8–1.0 μm in diameter and 10–60 μm in length, found mostly within the floc. Cells are rectangular (0.8 × 1.5 μm) without constrictions at septa, or with constrictions that are hard to observe. Neither attached growth nor a sheath is present. Cells stain Gram negative and the entire trichome stains Neisser positive (purple). No sulfur granules.

    This filament often is overlooked, or its abundance underestimated, until the Neisser stained slide is examined. Filaments appear wider (1.0–1.2 μm) in dried, stained smears.

13. *Type 0961.* (Figure 33a and b.) Straight trichomes, 0.8–1.4 μm in diameter and 50–150 μm in length, observed extending from the floc surface. No attached growth. Cells are rectangular (0.8–1.4 × 1.5–4 μm). A true sheath is not present; however, a slime coating may be present, appearing as an empty "cuff" at the trichome apex. Gram negative and Neisser negative and no sulfur granules. Cells appear "transparent" without any intracellular contents.

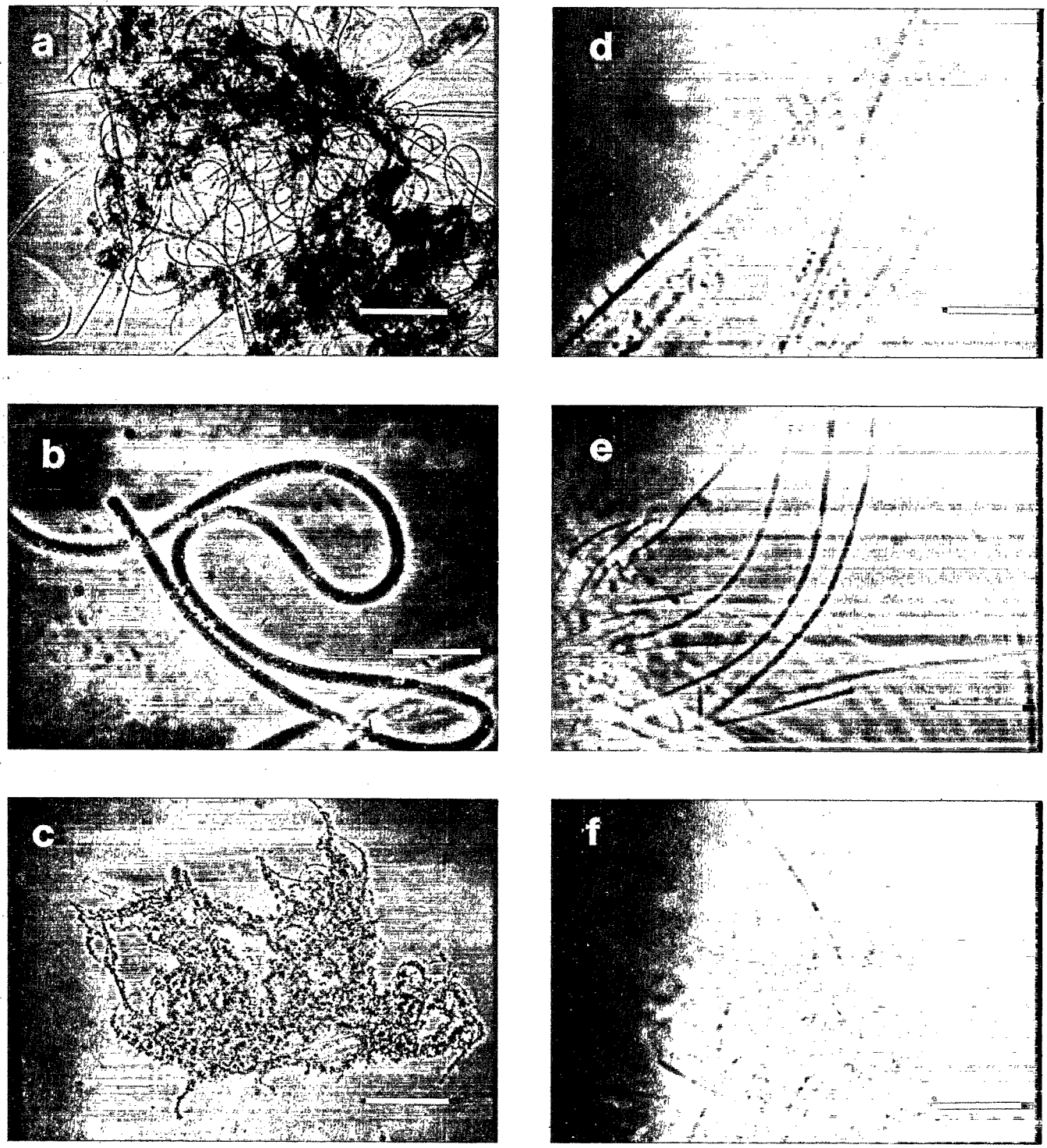
14. *Microthrix parvicella.* (Figure 33c, d, and e; see also Figures 23e and 24e.) Irregularly-coiled filaments, 0.6–0.8 μm in diameter and 100–400 μm in length, found in tangles in the floc or as loose "patches" free in the bulk solution. Neither attached growth nor a sheath is present. No branching. Cell septa are not observed; however, substantial intracellular granules may occur, giving a "beaded" effect. Gram-positive, Neisser-negative, and Neisser-positive granules commonly occur. Short, clear spaces may occur in the filament.

15. *Nocardia* spp. (Figure 34a, b, and c; see also Figures 16b, 23f, and 24f.) Irregularly-bent, short filaments, 1.0 μm in diameter and 10–20 μm in length, found mostly within the floc, but may occur free in the bulk solution. A branched (true branching) mycelium often is observed. No sheath and no attached growth. Cell shape is somewhat irregular (1.0 × 1.0–2.0 μm) and septa without constrictions are present. Gram positive and Neisser negative, and Neisser-positive granules commonly are observed. No sulfur granules. PHB commonly is observed. Abundance of this organism is best assessed from the Gram stained preparation.

16. *Nostocoida limicola* I. Bent and irregularly-coiled filaments, 0.6–0.8 μm in diameter and 100–200 μm in length, found within the flocs and free in the bulk solution. Cell septa are hard to observe; when observed, cells are oval (0.6–0.8 μm diameter). No sheath and no attached growth. Gram positive and Neisser-positive trichome. No sulfur granules. Resembles *M. parvicella,* except in Neisser-staining properties.

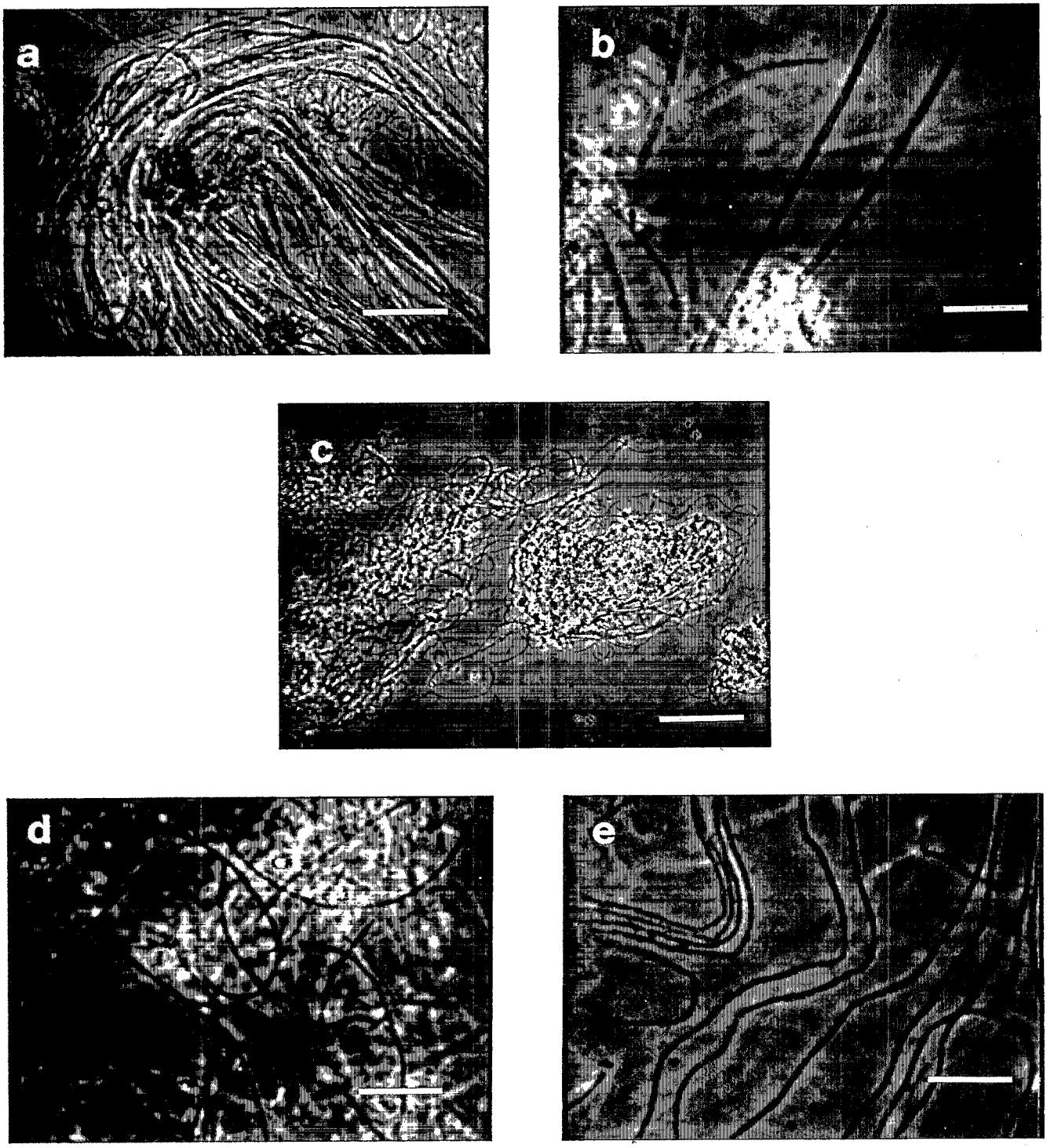
17. *Nostocoida limicola* II. (Figure 34d, e, and f; see also Figures 16c and 24d.) Bent and irregularly-coiled filaments, 1.2–1.4 μm in diameter and 100–200 μm in length, found mostly within the floc. Cell septa are clear, with oval cells (1.2–1.4 μm in diameter) and indentations at septa. No sheath and no sulfur granules. PHB granules commonly are observed. Gram and Neisser staining reactions are variable. Most observed is Gram negative, but a Gram-positive reaction occurs. Most often the entire trichome stains Neisser positive (purple), but can be Neisser negative at times. Incidental branching is observed. *Note:* Eikelboom and van Buijsen (1981) state that *N. limicola* II is both Gram positive and Neisser negative, and that a bacterium closely resembling *N. limicola* II occurs in some industrial waste activated sludge systems, differing from *N. limicola* II by being Gram negative and Neisser negative. Both forms are considered *N. limicola* II here.

Figure 32. *Beggiatoa* spp. (*a* and *b*), type 1851 (*c* and *d*), type 0803 (*e*) and type 0092 (*f*) (*a* and *c* 100× phase contrast; bar = 100 μm; *b*, *d*, *e* and *f* 1000× phase contrast; bar = 10 μm).



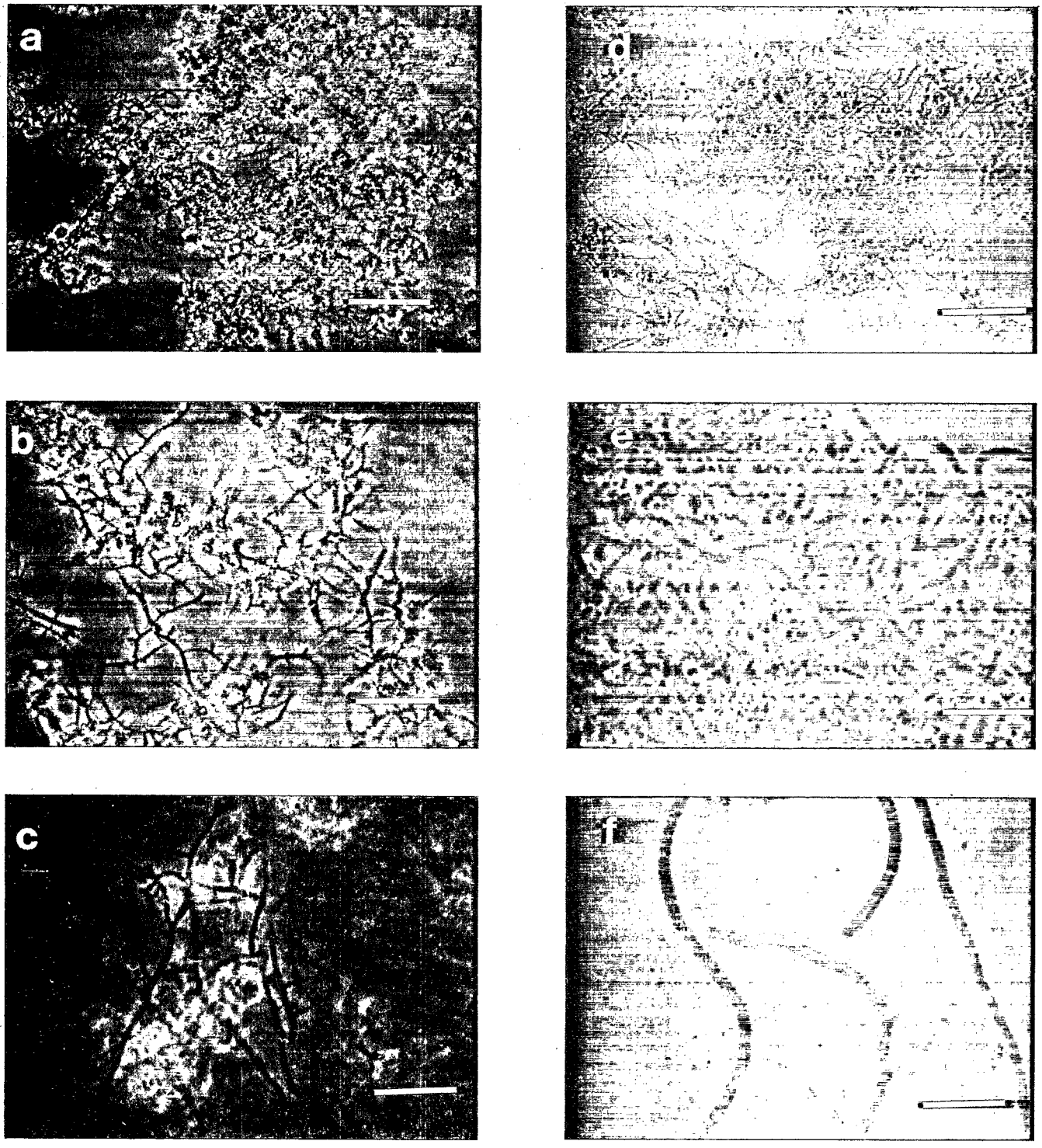

Reference: Jenkins, *et al.*, 1984.

Figure 33. Type 0961 (*a* and *b*) and *Microthrix parvicella* (*c, d* and *e*) (*a* and *c* 100× phase contrast; bar = 100 μm; *b, d* and *e* 1000× phase contrast; bar = 10 μm).




Reference: Jenkins, *et al.*, 1984.

Figure 34. *Nocardia* spp. (*a, b* and *c*) and *Nostocoida limicola* II (*d, e* and *f*) (*a* and *d* 100× phase contrast; bar = 100 μm; *b* 400× phase contrast; bar = 25 μm; *c, e* and *f* 1000× phase contrast; bar = 10 μm).




Reference: Jenkins, *et al.*, 1984.

18. *Nostocoida limicola* III. Bent and irregularly-coiled filaments, 1.6–2.0 μm in diameter and 200–300 μm in length, found mostly within the floc. No attached growth and no sheath. Cell septa are clear, with oval cells (1.6–2.0 μm diameter) and indentations at cell septa. PHB granules are commonly observed. Gram positive and Neisser positive. No sulfur granules.

19. *Haliscomenobacter hydrosis.* (Figure 35a, b, and c.) Straight or bent, thin filaments, 0.5 μm in diameter and 20–100 μm in length, found radiating outward from the floc surface or free in the bulk solution. A sheath is present. No cell septa are observed; however, empty spaces in the trichome are commonly observed. Gram negative and Neisser negative. No sulfur granules. Filaments may occur in bundles, and attached growth is variable, ranging from rare to abundant. This filament can be easily overlooked at 100× observation.

20. *Type 0581.* (Figure 35d.) Smoothly-coiled filaments, 0.4–0.7 μm in diameter and 100–200 μm in length, found mostly within the floc but may occur in "patches" free in solution. No sheath and no cell septa. No sulfur granules; no attached growth. Gram negative and Neisser negative. This filamentous microorganism appears similar to *M. parvicella,* but differs in its Gram and Neisser staining reactions.

21. *Type 1863.* (Figure 35e.) Short, irregularly-bent filaments, 0.8 μm in diameter and 50μm in length, found extending from the floc surface and free in the bulk solution. No sheath and no attached growth. Cells are oval-shaped rods (0.8 × 1.5 μm), and appear as a "chain of cells," with indentations at the septa. Gram negative and Neisser negative; Neisser-positive granules may occur. No sulfur granules.

22. *Type 0411.* (Figure 35f.) Irregularly-bent trichomes, 0.8 μm in diameter and 50–150 μm in length, found extending from the floc surface. Filaments composed of elongated, rod-shaped cells (0.8–2.4 μm); constrictions at septa give the appearance of a "chain of cells." No sheath and no attached growth. Gram negative and Neisser negative. No sulfur granules.

23. *Type 1702.* (Figure 36a.) Short, straight or bent filaments, 0.6–0.7 μm in diameter and 20–80 μm in length, found within the floc and extending from the floc surface. Cell septa are absent and a sheath is present. Incidental attached growth. Gram negative and Neisser negative. No sulfur granules.

24. *Type 1852.* (Figure 36b.) Straight or slightly bent filaments, 0.6–0.8 μm in diameter and 20–80 μm in length, found extending from the floc surface. Cells are rectangular (0.6–0.8 × 1.0–2.0 μm) without constrictions at septa. No sheath, no attached growth. Gram negative and Neisser negative. No sulfur granules. This filament appears "transparent," as does type 0961.

25. *Type 0211.* (Figure 36c.) Bent and twisted filaments, 0.3–0.5 μm in diameter and 20–100 μm in length, found extending from the floc surface. Cells are rod-shaped with clear constrictions at cell septa. No sheath and no attached growth. Gram negative and Neisser negative. No sulfur granules.

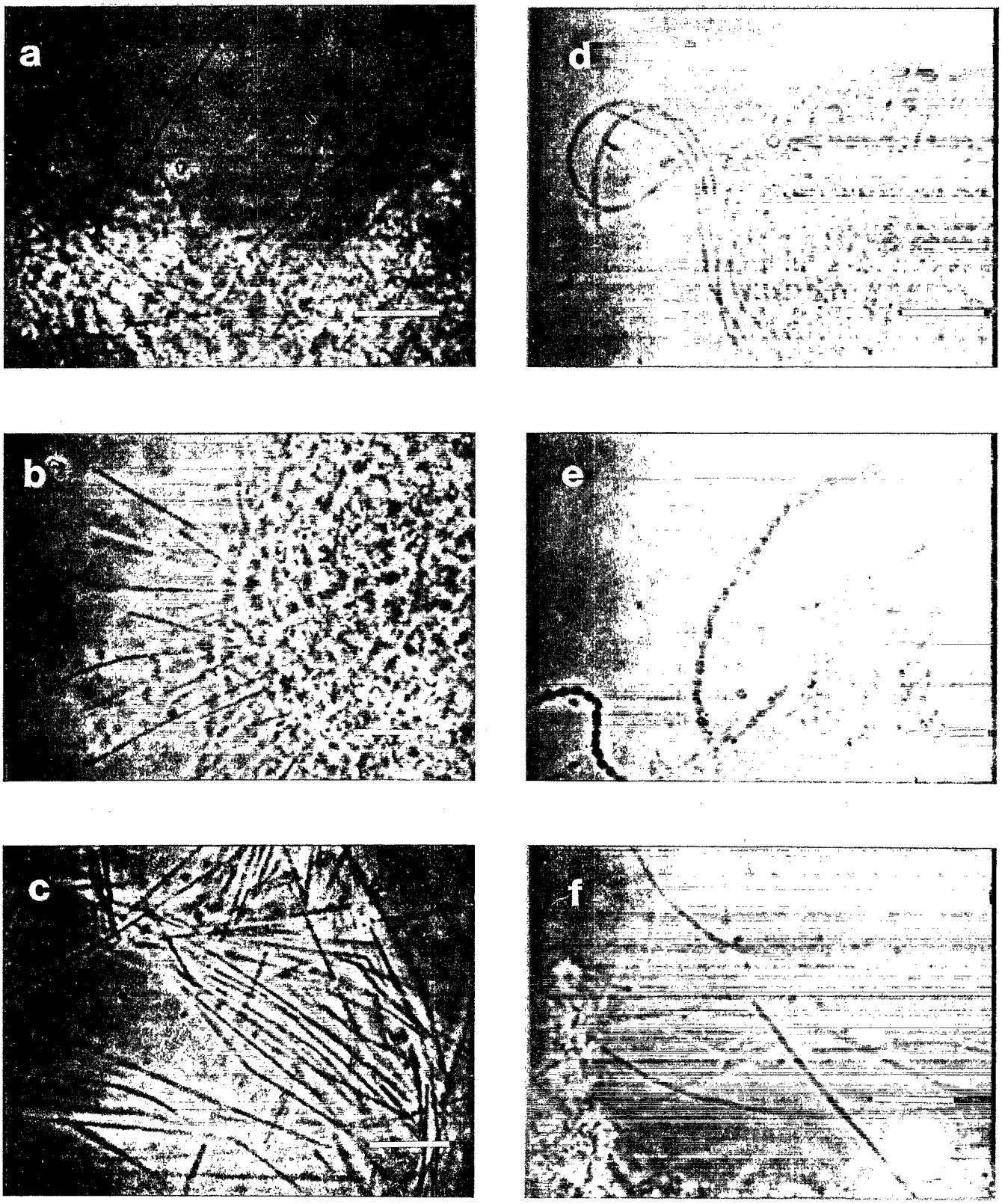
26. *Flexibacter* spp. (Figure 36d.) Short, straight or smoothly-curved filaments, 1.0 μm in diameter and 20–40 μm in length, found free in the bulk solution. This organism is motile by slow gliding and flexing. No sheath and no attached growth. Cell septa may be lacking. Gram negative and Neisser negative. PHB granules commonly observed.

27. *Bacillus* spp. (Figure 36e.) Rounded-end rods in irregularly-shaped chains of cells, 0.8–1.0 μm in diameter and 20–50 μm in length, found mostly at the edges of the floc. Gram positive and Neisser negative. No sheath and no sulfur granules.

28. *Cyanophyceae.* (Figure 36f.) Straight, large filaments, 2.0–5.0 μm in diameter and 100–500 μm in length, found free in the bulk solution. Cells are square to rectangular (2–5 × 2–8 μm) with clear septa. No sheath and no attached growth. Often motile by slow gliding. Distinct green color under transmitted light observation. Gram negative but sometimes with a slight Gram-positive reaction; Neisser negative. No sulfur granules.

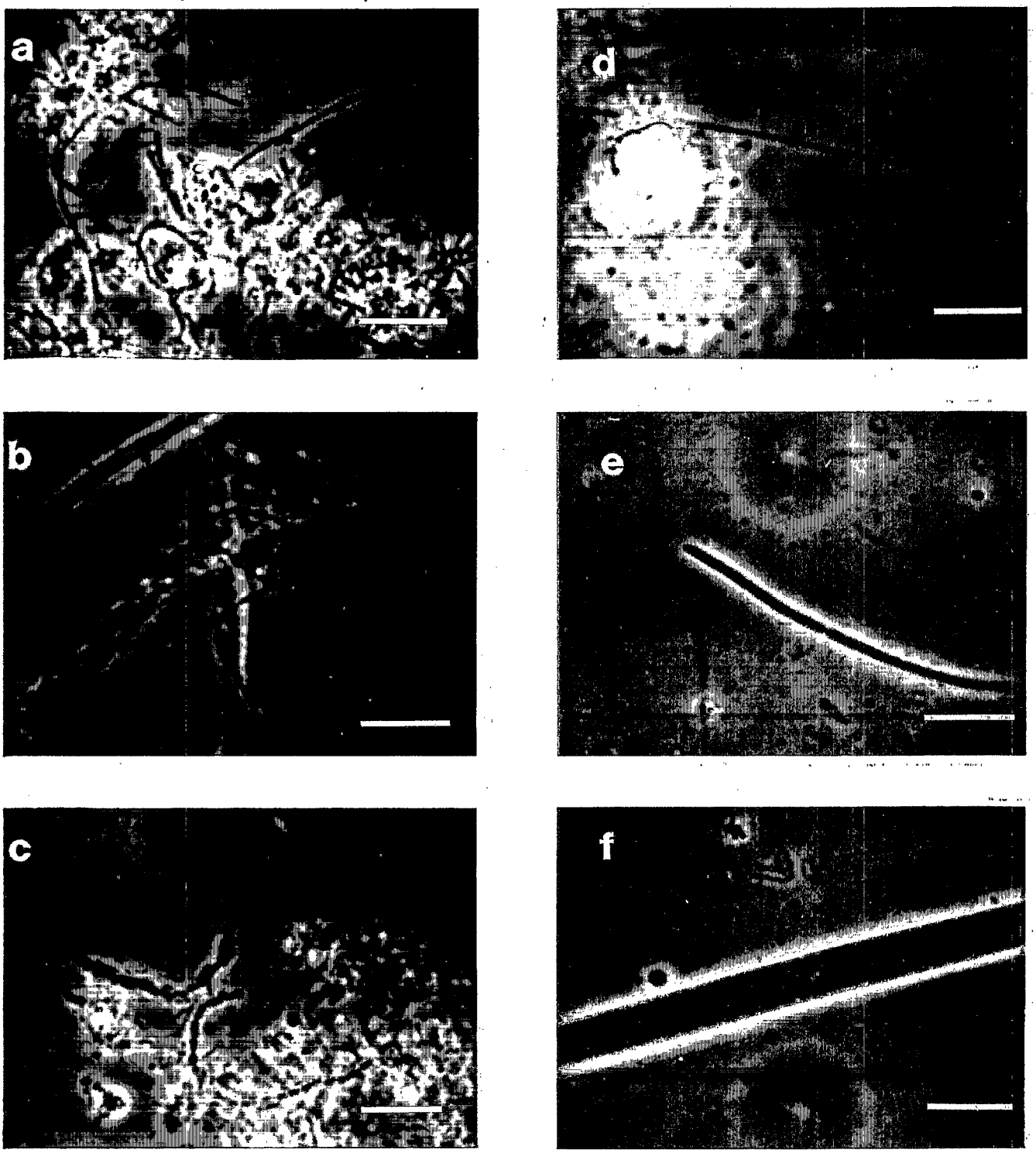
29. *Fungi.* (Figure 37a, 37b, and 37c; see also Figure 16a.) Very large trichomes, 3–8 μm in diameter and 300–1000 μm in length, found mostly in the floc. Cells are rectangular (3–8 × 5–15 μm) and contain intracellular granules and organelles; cytoplasmic streaming may be observed. True branching. Gram negative and Neisser negative. No sulfur granules and no sheath, although a heavy cell wall is present.

Figure 35. *Haliscomenobacter hydrossis* (*a, b* and *c*), type 0581 (*d*), type 1863 (*e*) and type 0411 (*f*) (all 1000× phase contrast; bar = 10 $\mu$m).



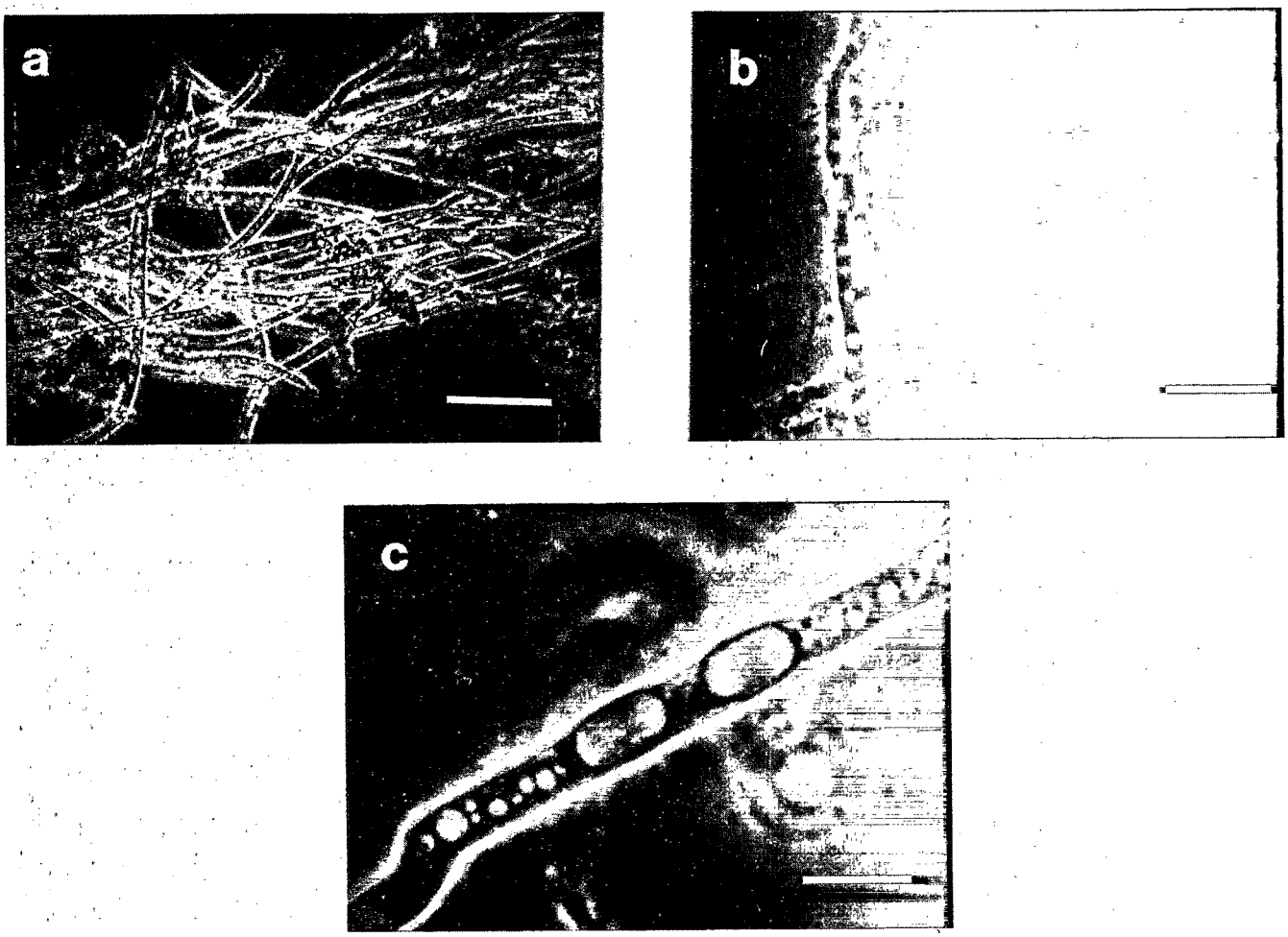

Reference: Jenkins, *et al.*, 1984.

Figure 36. Type 1702 (*a*), type 1852 (*b*), type 0211 (*c*), *Flexibacter* sp. (*d*), *Bacillus* sp. (*e*) and a blue-green bacterium (cyanophyceae) (*f*) (all 1000× phase contrast; bar = 10 $\mu$m).




Reference: Jenkins, *et al.*, 1984.

Figure 37. Fungus (*a* 100× phase contrast; bar = 100 μm; *b* 400× phase contrast; bar = 25 μm; *c* 1000× phase contrast; bar = 10 μm).




Reference: Jenkins, *et al.*, 1984.

## Chapter 7

## 7.0 Appendix 3—Case Histories of Bulking Control Using Chlorination.

*City of Albany, GA.* The City of Albany, GA, is a 20-MGD (0.88 $m^3$/sec) (design) activated sludge plant currently receiving 13 to 16 MGD (0.57 to 0.7 $m^3$/sec) of a waste in which approximately 50 percent of the $BOD_5$ and SS loads are from industry (paper processing and convenience foods manufacturing). The plant experienced bulking problems to such a degree that, with only approximately 12 MGD (0.5 $m^3$/sec) of influent flow, attainment of secondary effluent criteria (30 mg/l monthly average for $BOD_5$ and SS) was not possible.

At Albany, the permanent RAS chlorination system injects chlorine solution into a series of well-mixed RAS wet wells. The installation consists of a Wallace and Tiernan Chlorinator 2,200 lb/day (1,000 kg/day) capacity, and a 2-inch (50mm) injector with 2-inch (50mm) PVC injector water and chlorine solution lines. The chlorine injection system is enclosed in a sheet metal housing for protection. The chlorine solution line is a 2-inch (50mm) PVC line leading to a 16-foot (5m) long, 2-inch (50mm) PVC header with 1/4-to 3/8-inch (6 to 10mm) diameter holes at 4-inch (100mm) spacing. The system was constructed in November 1977 for $4,953.

Figure 38 presents the history of RAS chlorination at Albany. When chlorination for bulking control was initiated, the RAS chlorination system did not exist. Because of the urgency of controlling SVI, chlorine was added to a wet well where the entire RAS stream mixed with primary effluent. This mode of chlorination was commenced in Week 15 and continued until Week 26, when the change to RAS chlorination was made. Chlorine was being added to the mixture of RAS and primary effluent, and doses of 5 to 15 lbs $Cl_2/10^3$lbs (5 to 15 kg $Cl_2/10^3$kg) SS/day were needed to control bulking. Immediately after the change from chlorinating the mixture of RAS and primary effluent to chlorinating RAS alone, chlorine doses that satisfactorily controlled bulking for the mixture of RAS and primary effluent were "overdoses" for RAS alone. Figure 38 shows that even though the chlorine dose declined during the changeover of the chlorine dose point [from 4.7 to 2.9 lbs $Cl_2/10^3$lbs (4.7 to 2.9 kg $Cl_2/10^3$kg) SS/day] there was an increase in secondary effluent SS (and to a lesser extent in secondary effluent $BOD_5$) that resulted from floc breakup caused by the chlorine overdose.

RAS chlorination has been practiced with generally excellent results at Albany for a period of over 7 years. At various times, the target SVI has been changed, and in some instances (Figure 38, Weeks 180 to 200), RAS chlorination was not initiated soon enough to prevent secondary effluent deterioration by a loss of sludge blanket solids in the effluent during peak flow periods. To illustrate the chlorine dosing technique for bulking control, as employed by the City of Albany, a bulking incident is examined in detail in Figure 39. At this time, the target SVI was 230 ml/g. The chlorine dose was started at low levels and gradually increased until the SVI responded by stabilizing and then falling. In the middle of this period (30 May through 6 June), the SVI fell to below the target value. The RAS chlorine dose was reduced in response to this. On 18–20 June, the SVI dropped to approximately 100 to 130 ml/g. The chlorine dose to the RAS was reduced and then turned off.

*City of San Jose/Santa Clara Water Pollution Control Plant (SJ/ SC WPCP), CA.* The SJ/SC WPCP provides tertiary treatment to 100 MGD (4.4 $m^3$/sec) of domestic sewage; during July–September (the peak load season), flows increased approximately 20% and $BOD_5$ loading approximately doubled due to cannery waste discharges. Effluent discharge criteria include 30-day average $BOD_5$ and SS of 10 mg/l each and receiving water undissociated ammonia standards that dictate virtually complete nitrification. The plant has two stages of activated sludge with complete nitrification and tertiary effluent filtration. It had plant upsets due to bulking in the secondary activated sludge system during the peak load seasons of 1979 and 1980. Interim measures included RAS chlorination in both the secondary and tertiary activated sludge systems, and provision of supplemental oxygen and ammonia to prevent bulking in the 1981 and 1982 peak load seasons (Beebe *et al.*, 1982).

The importance of chlorine solution mixing into the RAS for providing effective filamentous microorgan-

ism control was illustrated at the SJ/SC WPCP. In the first-stage activated sludge system, the initial chlorine injection point to the RAS was through a PVC pipe with a 4-foot (1.2m) freefall into a 20 x 16 x 13 foot (6m x 5m x 4m) wet well. It soon became evident that this arrangement was ineffective. Extension of the 2-inch (75 mm) PVC pipe to a point 5 feet (1.5m) below the RAS surface in this wet well virtually eliminated loss of gaseous chlorine, but SVI control was still inefficient. Doses of 8 to 10 lbs $Cl_2/10^3$lbs (8 to 10 kg $Cl_2/10^3$kg) SS/day had little effect on SVI but created a turbid secondary effluent.

Following this failure, two alternate RAS chlorination devices were installed:

(i) Diffusers about mid-depth across the full width of the aerated mixed liquor channels leading to the secondary clarification tanks;

(ii) Single outlet injectors through the clean-out ports about 6 inches (15 cm) upstream of closed-impeller, low-speed centrifugal RAS pumps.

The data presented in Figure 40 show that the pump clean-out port injection point, with its superior mixing, provided more rapid, predictable, and efficient SVI control. Operating data indicated that approximately 20 times more chlorine was added when chlorine was dosed to the RAS well rather than to the RAS pump clean-out port. As a result of this success, a similar chlorine injection system was installed in the second-stage activated sludge system. It too has been used successfully for bulking control without compromising the nitrifying ability of the second-stage activated sludge system. Injection of chlorine solution into the first- and second-stage activated sludge system RAS pumps for over 3 years has had no deleterious effects on these pumps, even though the second-stage RAS pumps have brass bearings. Impellers in all RAS pumps are cast iron, and great care is taken to make certain that chlorine solution is never fed to an out-of-service pump.

RAS chlorination for control of filamentous bulking has been developed to a high degree at the SJ/SC WPCP (Figure 41). Chlorine doses are regulated routinely on the basis of a target SVI value with input from the plant microbiologist who observes activated sludge samples on a daily basis during periods of RAS chlorination. During past peak load seasons, extremely low target SVI values (60 to 80 ml/g) have been used in the first stage activated sludge system so that high solids loading rates (30–50 lbs SS/ft.$^2$/day) (150–250 kg SS/m$^2$/day) could be applied to the secondary clarifier. To maintain SVI values this low, chlorine doses to the first-stage activated sludge system often were high—in the range of 8 to 16 lbs $Cl_2/10^3$lbs (8 to 16 kg $Cl_2/10^3$kg) VSS/day. These high chlorine doses produced significant floc destruction, resulted in turbid secondary effluents, and caused increases in secondary effluent dissolved total organic carbon (TOC) concentrations. The elevated turbidity and TOC increase from approximately 15 to 35 mg/l could be tolerated at the SJ/SC WPCP, because the downstream second-stage activated sludge system readily polishes the effluent. Applying such high chlorine doses and obtaining a high-quality secondary effluent would be difficult if the first-stage activated sludge plant were operated without the second-stage activated sludge system to capture the fine solids.

RAS chlorination also is effective for bulking control in the second-stage (nitrifying) activated sludge system (Figure 42). At the chlorine doses used [2–4 lbs $Cl_2/10^3$lbs (2-4 kg $Cl_2/10^3$kg) VSS/day]; 1.5 to 3.0 mg $Cl_2$/l dose in the RAS stream), nitrification efficiency was unaffected. This observation is consistent with that of Strom and Finstein (1977). In the nitrifying system, the decrease in SVI with commencement of RAS chlorination was much faster than it was in the first-stage activated sludge system. Whether this was due to the presence of the more germicidal free chlorine in the nitrifying system, to differences in the types of filamentous microorganisms causing bulking (type 0041 in the second stage, type 1701 in the first stage), or to differences in system sludge growth rates is not known.

*Case history of bulking control using hydrogen peroxide. City of Petaluma, CA (Caropresso et al., 1974).* During 1974, the City of Petaluma, CA, Water Pollution Control Plant, which treated mixed domestic/industrial wastes, experienced severe bulking problems with SVI values in the range of 400 to 700 ml/g. Hydrogen peroxide (as a 50% V/V $H_2O_2$ solution) was dosed to the final quadrant of a two-pass aeration tank that was being fed primary effluent at the 1/4 points and RAS at the head end. Figure 43 shows the doses and concentrations of hydrogen peroxide used over a 4-day period and the effect on SVI. The hydrogen peroxide (100% as $H_2O_2$) doses ranged between 9-68 mg/l and were variously applied for 8-24 hr/day. During the dosing of hydrogen peroxide, 2300 lb (1050 kg) $H_2O_2$ (100% basis) was used to bring the SVI under control (from 550 ml/g to 300 ml/g). The SVI continued to decrease following the termination of hydrogen peroxide dosing.

Figure 38. Control of bulking by RAS chlorination at the City of Albany, GA, Wastewater Treatment Plant (Jenkins *et al.*, 1983).

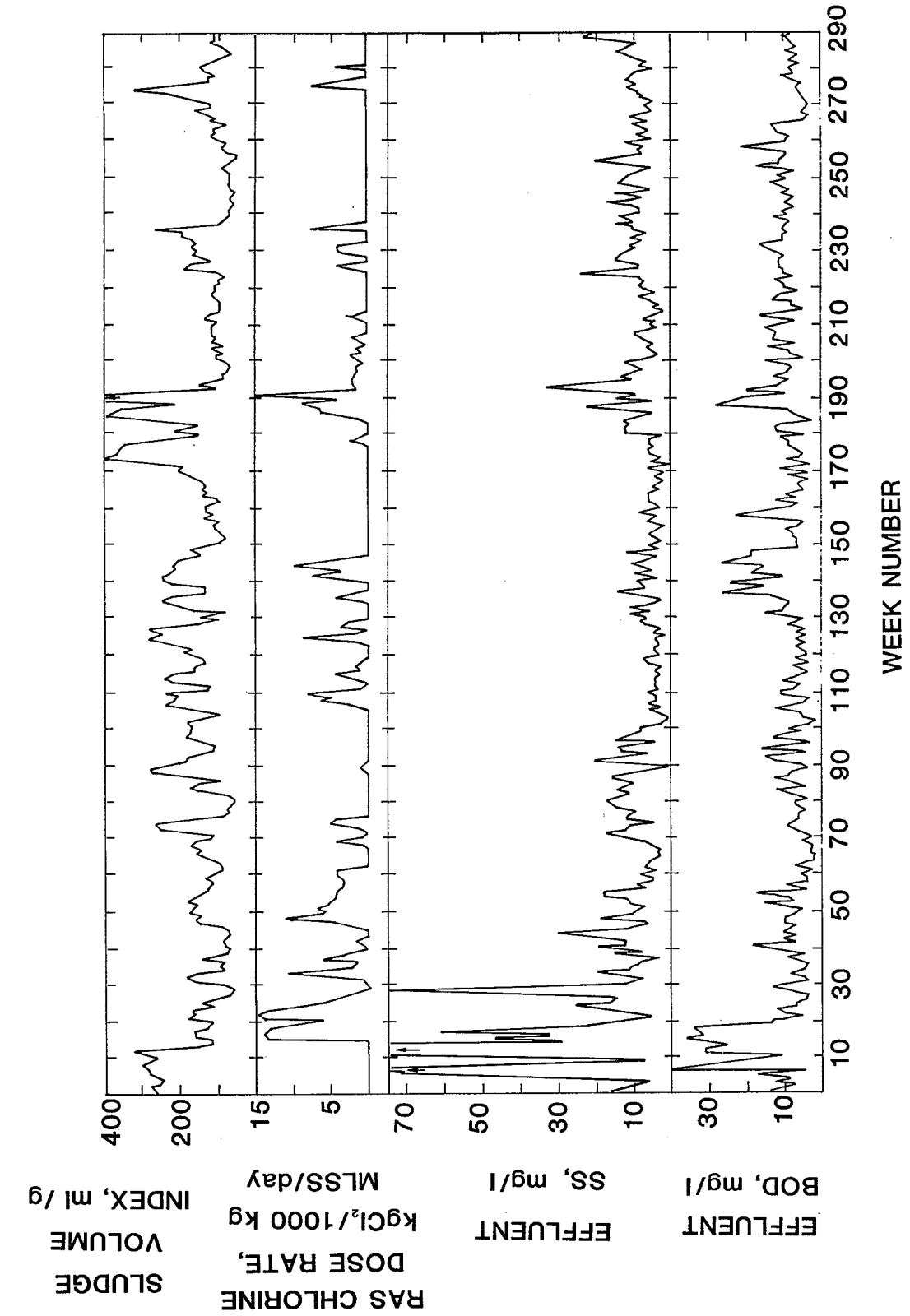


Figure 39. Use of target SVI to control RAS chlorination dosage for bulking control at the Albany, GA, Wastewater Treatment Plant (Jenkins, 1980).

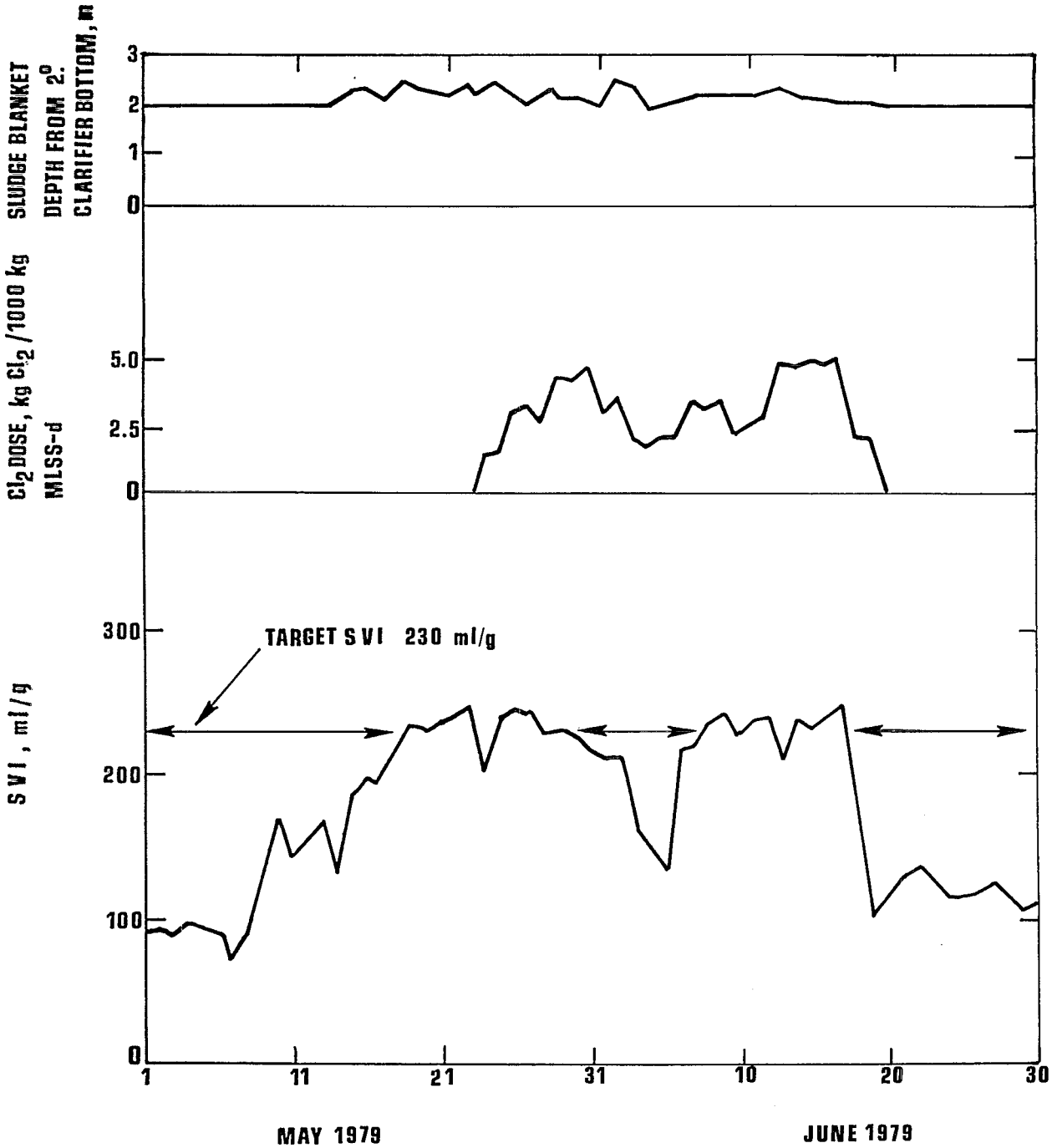


Figure 40. Comparison of RAS chlorination effectiveness when dosing chlorine to either the mixed liquor channel or the RAS pump (Beebe *et al.*, 1982).

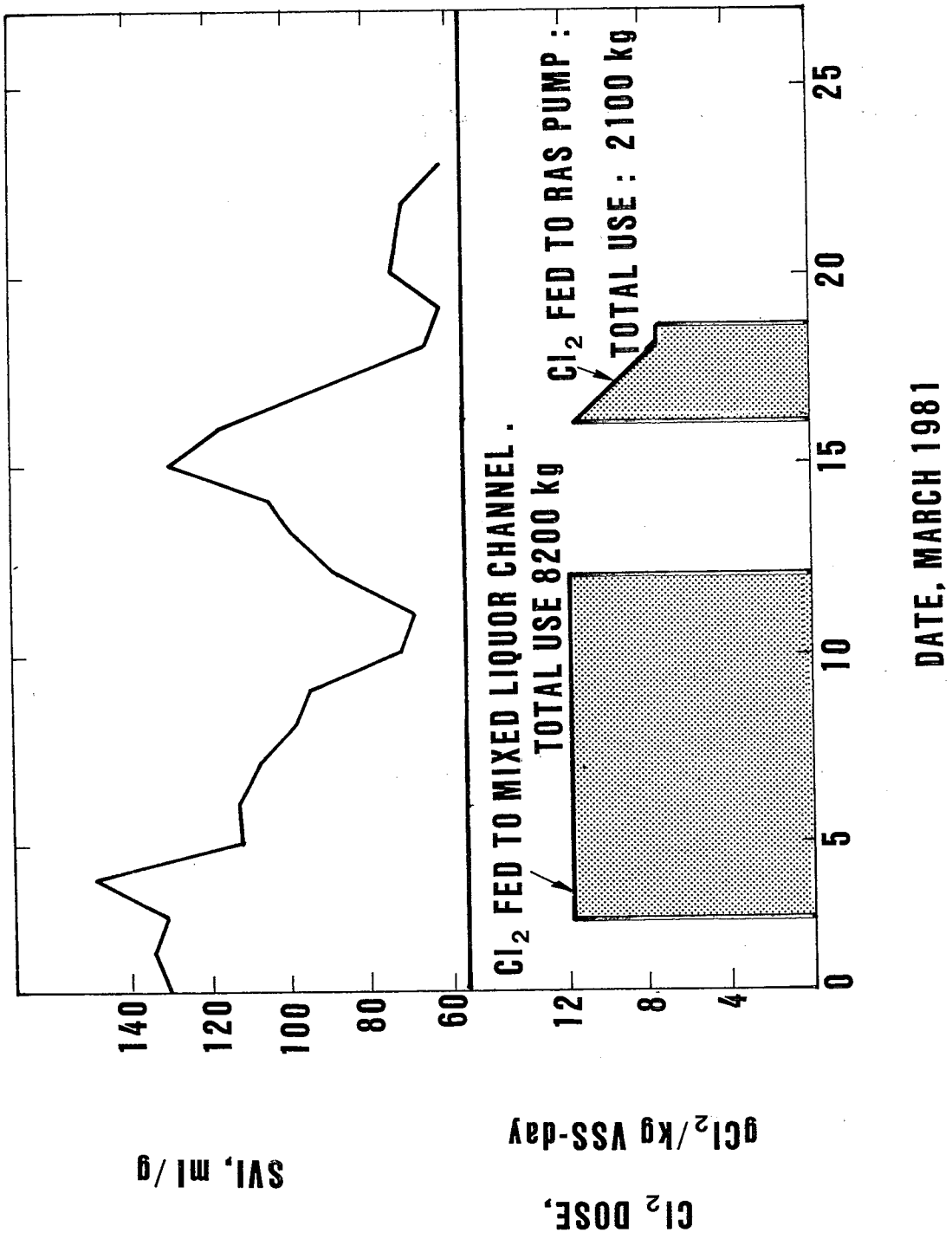


Figure 41. SVI and chlorine dose to RAS in the two first-stage activated sludge systems at the San Jose/Santa Clara Water Pollution Control Plant, CA—1981 peak load season (Beebe *et al.*, 1982).

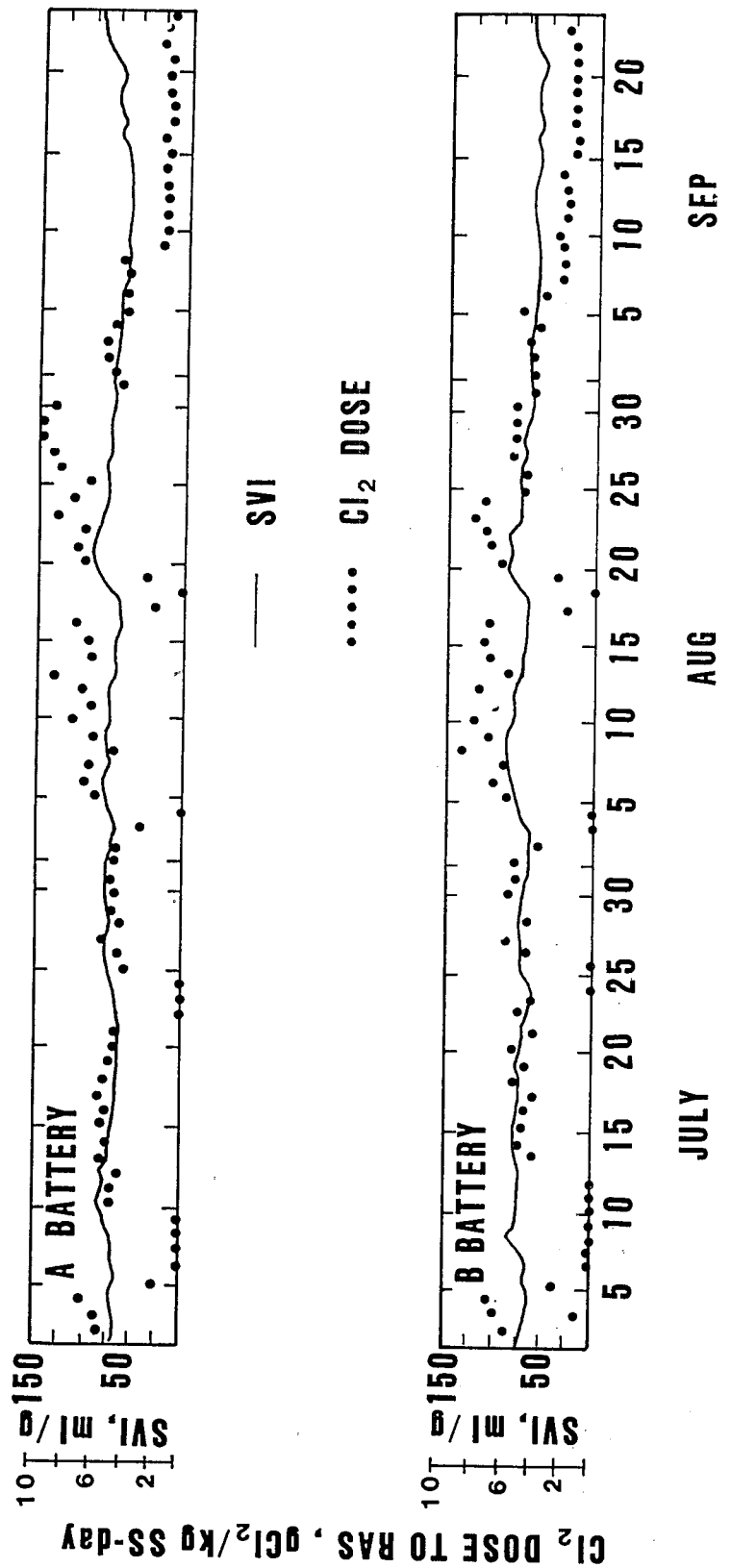


Figure 42. SVI and chlorine dose to RAS in the two second-stage activated sludge systems at the San Jose/Santa Clara Water Pollution Control Plant, CA—1981 peak load season (Beebe *et al.*, 1982).

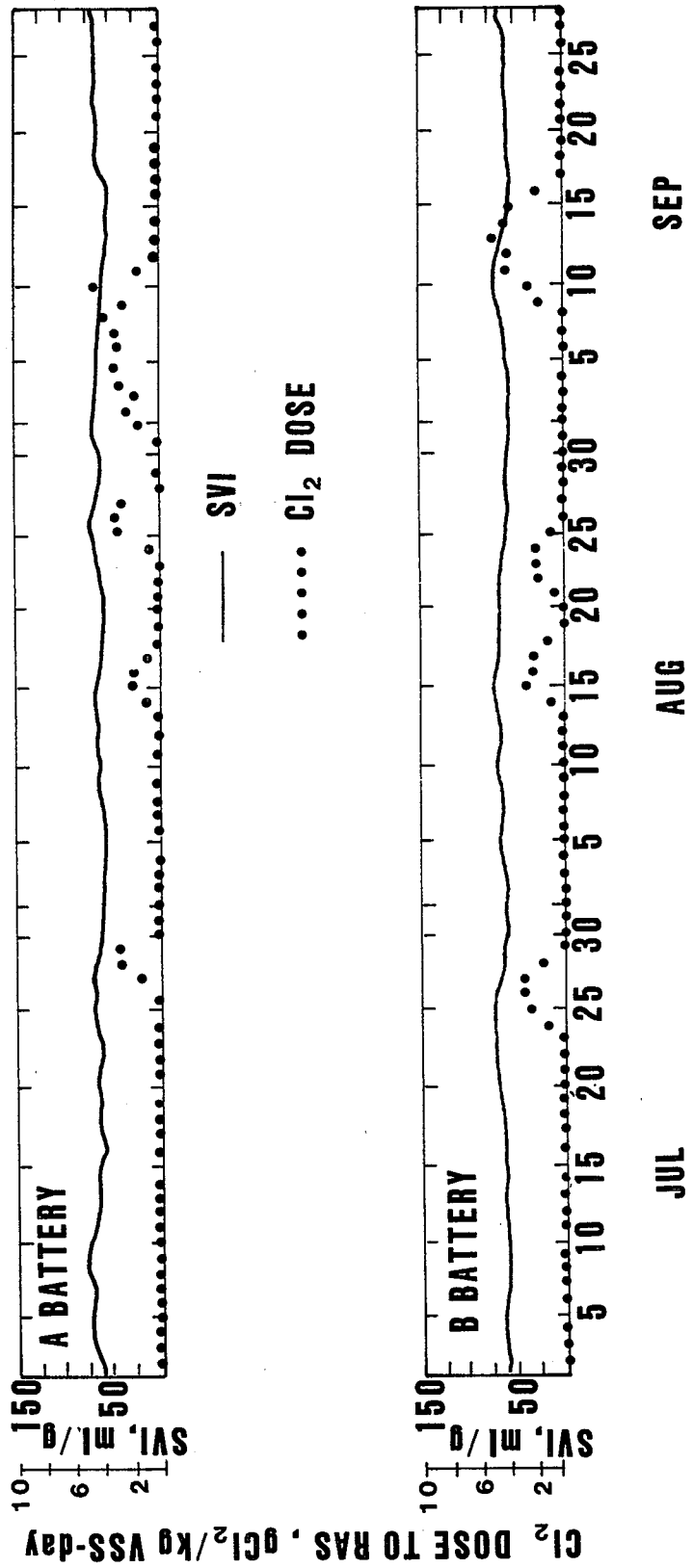
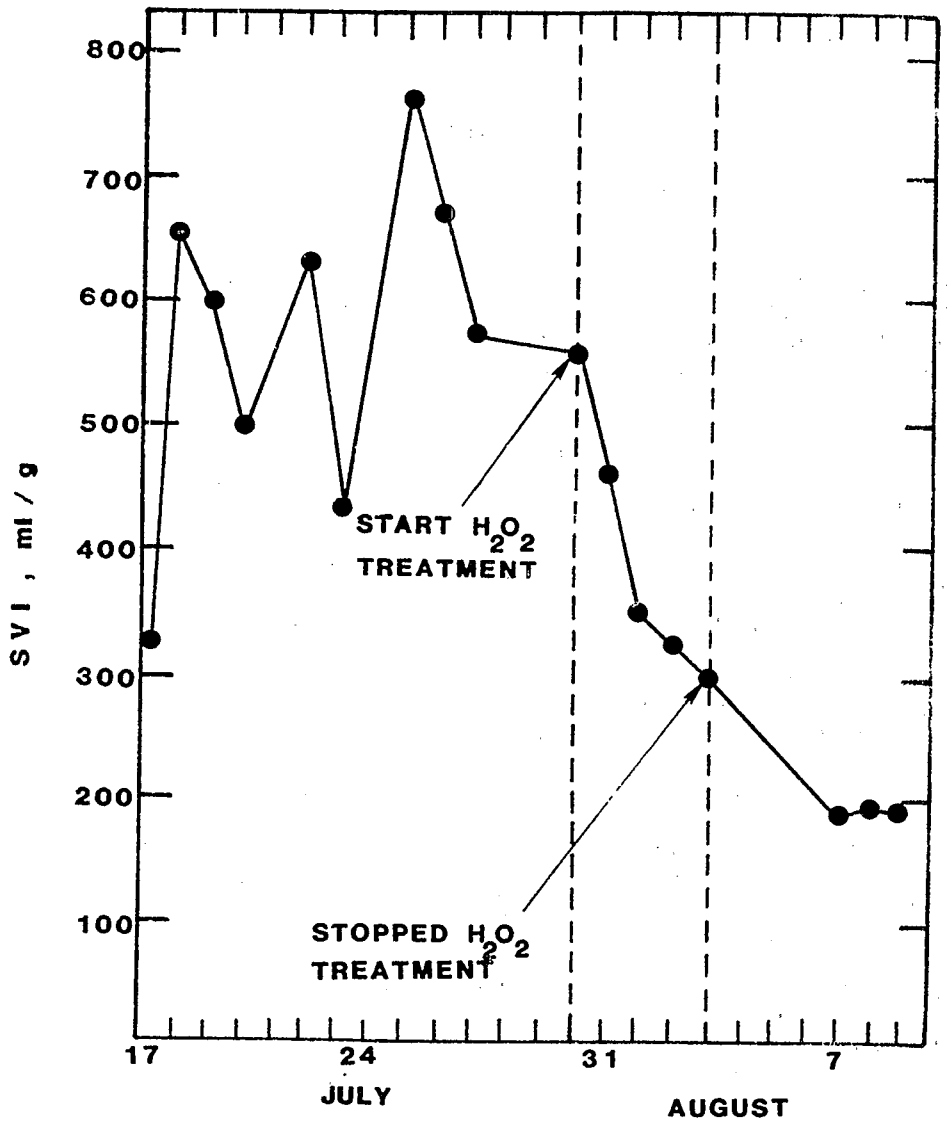


Figure 43. SVI response to peroxide treatment at the Petaluma, CA Water Pollution Control Plant (Caropreso *et al.*, 1974).



## *References*

This section, besides containing references cited in the text, also contains a more complete coverage of the literature on activated sludge bulking, filamentous organism growth and *Nocardia* growth and foaming.


Al-Diwamy, L.J. and Cross, T. (1978), "Ecological Studies of Nocardia Foams and Other Actinomycetes in Aquatic Habitats." In. *Nocardia and Streptomyces;* M. Modarski, W. Kurytowicz, and J. Jeljaszewicz (Eds.) Gustav Fischer Verlag, Stuttgart, Germany, 153.

Anon. (1969) "Milwaukee Mystery" Unusual Operating Problem Develops." *Water and Sew. Works, 116,* 213.

Anon. FMC Corporation (1973) "Bulking Control with Hydrogen Peroxide: Case History, Water Pollution Control Plant, City of Petaluma, Sonoma, CA." *Technical Data Polln. Control Release No. 41.*

Anon. (1974) *Bergey's Manual of Determinative Bacteriology* 8th Ed., R.E. Buchanan and N.E. Gibbons, Eds. The Williams and Wilkins Co., Baltimore.

Anon. FMC Corporation (1976) "Sludge Bulking Cure: Hydrogen Peroxide." *Polln. Control Release No. 95. Municipal South,* Nov./Dec.

Anon. (1979) "Hydrogen Peroxide Solves Bulking Problem at Coors' Waste Treatment Plant." *Food Engineering,* November 1979 and FMC Corporation *Technical Data Polln. Control Release No. 117.*

Anon. (1981) *Manual of Methods for General Bacteriology.* American Society for Microbiology, Washington, D.C.

Anon. (1983) "Thames Water Uses Chlorine to Control Bulking Sludge." *Water Research News, 10,* 6, Water Research Centre, Medmenham, Bucks, England.

Adamse, A.P. (1968) "Bulking of Dairy Waste Activated Sludge." *Water Research, 2,* 715.

Albagnac, G., and Morfaux, J.N. (1980) "Traitabilitie Comparee en Aeration Prolongee et en Contact-Stabilisation des Eau Residuaires de Brasierie." *Trib. Cebedeau, 33,* 63.

Allen, L.A. (1944) "The Bacteriology of Activated Sludge." *J. Hyg., 43,* 424.

Ardern, E. and Lockett, W.T. (1914*a*) "Experiments on the Oxidation of Sewage Without the Aid of Filters." *J. Soc. Chem. Ind., 33,* 523.

Ardern, E. and Lockett, W.T. (1914*b*) "The Oxidation of Sewage Without the Aid of Filters." *J. Soc. Chem. Ind., 33,* 112.

Banoub, A. (1982) "Reducing Energy Consumption: How 2 Communities Did It; Woonsocket, R.I." *Deeds and Data; Water Polln. Control Fedn. Highlights, 14,* 11.

Barnard, J.L. (1978) "Solving Sludge Bulking Problems." *Water Polln. Control, 77*(1), 103.

Barnes, D. and Goronszy, M.C. (1980) "Continuous Intermittent Wastewater Systems for Municipal and Industrial Effluents." *Public Health Engineer, 8,* 20.

Becker, J.G. and Shaw, C.G. (1955) "Fungi in Domestic Sewage Treatment Plants." *Appl. Microbiol, 3,* 173.

Beebe, R.D. and Jenkins, D. (1981) "Control of Filamentous Bulking at the San Jose/Santa Clara Water Pollution Control Plant." Presented at the 53rd Annual Conference, Calif. Water Polln. Control Assoc., Long Beach, CA.

Beebe, R.D., Jenkins, D. and Daigger, G.T. (1982) "Activated Sludge Bulking Control at the San Jose/Santa Clara, California Water Pollution Control Plant." Presented at the 55th Annual Conference of the Water Polln. Control Fedn., St. Louis, MO.

Beebe, R.D. (1983) Privately Communicated. San Jose/Santa Clara Water Pollution Control Plant, San Jose, CA.

Blackbeard, J.R. and Ekama, G.A. (1984) "Preliminary Report on Filamentous Micro-organisms Responsible for Bulking and Foaming in Activated Sludge Plants in Southern Africa." Dept. of Civil Engng., Univ. of Capetown, RSA.

Bode, H. (1983) "The Use of Chlorine for Bulking Control." Presented at the Institute fur Siedlungswasserwirtshaft, University of Hannover, FRG, 26 Jan. 1983.

Boyle, W.C. (1982) Discussion of Lee, S-E., Koopman, B.L., Jenkins, D. and Lewis, R.F. (1982) "The Interrelated Effects of Aeration Basin Configuration on Activated Sludge Bulking at Low Organic Loading." Presented at 10th Intl. Assoc. for Water Polln. Research and Control Conference, Capetown, South Africa.

Benefield, L.D., Randall, C. and King, P.H. (1975) "The Stimulation of Filamentous Micro-organisms in Activated Sludge by High Oxygen Concentrations." *Water Air and Soil Polln., 5,* 113.

Buhr, H.O. (1982) "Solids Distribution in a Step Feed Plant." Internal Report, J. Greeley and Hansen, Phoenix, AZ.

Butterfield, C.T. (1935) "Studies of Sewage Purification: II A Zoogloea-Forming Bacterium Isolated from Activated Sludge." *U.S. Pub. Health Rpts., No. 50,* 671.

Butterfield, C.T., Ruchoft, C.C. and McNamee, P.D. (1937) "Studies of Sewage Purification: VI Biochemical Oxidation by Sludges Developed by Pure Cultures of Bacteria Isolated from Activated Sludge." *U.S. Pub. Health Rpts. No. 52,* 387.

Caropreso, F.E., Raleigh, C.W., Brown, J.C. (1974) "Attack Bulking Sludge with $H_2O_2$ and a Microscope." *Bull., California Water Polln. Control Assoc.,* 44.

Carter, J.L. and McKinney, R.E. (1973) "Effects of Iron on Activated Sludge Treatment." *J. Environ. Engineer. Div. Amer. Soc. Civil Eng., 99, E2, Proceedings Paper 96-79,* 135.

Chambers, B. (1982) "Effect of Longitudinal Mixing and Anoxic Zones on Settleability of Activated Sludge." Chapter 10 in *Bulking of Activated Sludge: Preventative and Remedial Methods,* B. Chambers and E.J. Tomlinson, Eds. Ellis Horwood Ltd., Chichester, England.

Chiesa, S.C. and Irvine, R.L. (1982) "Growth and Control of Filamentous Microbes in Activated Sludge—An Integrated Hypothesis." Presented at the 55th Annual Conference of the Water Polln. Control Fedn., St. Louis, MO.

Chudoba, J., Ottova, V. and Madera, V. (1973*a*) "Control of Activated Sludge Filamentous Bulking. I. Effect of Hydraulic Regime or Degree of Mixing in an Aeration Tank." *Water Research, 7,* 1163.

Chudoba, J., Grau, P. and Ottova, V. (1973*b*) "Control of Activated Sludge Filamentous Bulking. II. Selection of Micro-organisms by means of a Selector." *Water Research, 7,* 1389.

Chudoba, J., Blaha, J. and Madera, V. (1974) "Control of Activated Sludge Filamentous Bulking. III. Effect of Sludge Loading." *Water Research, 8,* 231.

Chudoba, J., Dohanyos, M. and Grau, P. (1982) "Control of Activated Sludge Filamentous Bulking. IV. Effect of Sludge Regeneration." *Water Sci. and Technol., 14,* 73.

Cole, C.A., Stamberg, J.B. and Bishop, D.F. (1973) "Hydrogen Peroxide Cures Filamentous Growth in Activated Sludge." *J. Water Polln. Control Fedn., 45,* 829.

Cooke, W.B. and Ludzack, F.J. (1958) "Predaceous Fungi Behavior in Activated Sludge Systems." *Sewage and Ind. Wastes, 30,* 1490.

Cooke, W.B. and Pipes, W.O. (1970) "The Occurrence of Fungi in Activated Sludge." *Mycopath. Mycop. Appl., 40,* 249.

Curtis, E.J.C. (1969) "Sewage Fungus: Its Nature and Effects." *Water Research, 3,* 280.

Cyrus, Z. and Sladka, A. (1970) "Several Interesting Organisms Present in Activated Sludge." *Hydrobiologia, 35,* 383.

Daigger, G.T., Robbins, M.H., Jr. and Marshall, B.R. (1983) "The Design of a Selector to Control Low F/M Filamentous Bulking." Presented at the 50th Annual Conference, Water Polln. Control Fedn., Atlanta, GA.

Daigger, G.T. and Roper, R.E., Jr. (1984) "The Relationship Between SVI and Activated Sludge Settling Characteristics." In Press, *J. Water Polln. Control Fedn.*

Dhaliwal, B.S. (1979) "Nocardia Amarae and Activated Sludge Foaming." *J. Water Polln. Control Fedn., 51,* 344.

Dias, F.F. and Bhat, J.V. (1964) "Microbial Ecology of Activated Sludge. I. Dominant Bacteria." *Appl. Microbiol. 12,* 412.

Dias, F.F., Dondero, N.C. and Finstein, M.S. (1968) "Attached Growth of Sphaerotilus and Mixed Populations in a Continuous-Flow Apparatus." *Appl. Microbiol., 16,* 1191.

Dick, R.I. and Vesilind, P.A. (1969) "The Sludge Volume Index—What is it?" *J. Water Polln. Control Fedn., 41,* 1285.

Donaldson, W. (1982) "Some Notes on the Operation of Sewage Treatment Works." *Sewage Works, 4,* 48.

Dondero, N.C. (1961) "Sphaerotilus: Its Nature and Economic Significance." *Advances in Appl. Microbiol., 3,* 77. New York: Academic Press.

Dondero, N.C., Phillips, R.A. and Heukelekian, H. (1961) "Isolation and Preservation of Cultures of Sphaerotilus." *Appl. Microbiol, 9,* 219.

Eikelboom, D.H. (1975) "Filamentous Organisms Observed in Bulking Activated Sludge." *Water Research, 9,* 365.

Eikelboom, D.H. (1977) "Identification of Filamentous Organisms in Bulking Activated Sludge." *Prog. Water Technol., 8,* 153.

Eikelboom, D.H. and van Buijsen, H.J.J. (1981) *Microscopic Sludge Investigation Manual.* TNO Research Institute for Environmental Hygiene. The Netherlands.

Eikelboom, D.H. (1982*a*) "Biosorption and Prevention of Bulking Sludge by Means of a High Floc Loading." Chapter 6 in *Bulking of Activated Sludge: Preventative and Remedial Methods. B. Chambers and E.J. Tomlinson, Eds., Ellis Horwood Ltd., Chichester, England.*

Eikelboom, D.H. *(1982b)* "Biological Characteristics of Oxidation Ditch Sludge" in *Oxidation Ditch Technology,* Proceedings published by CEP Consultants Ltd., Edinburgh, Scotland.

Ekama, G.A. and Marais, G.V.R. (1985) *Technical Guide for the Design of Secondary Clarifiers for the Activated Sludge Process.* Water Research Commission, Pretoria, RSA.

Farquhar, G.J. and Boyle, W.C. (1971*a*) "Identification of Filamentous Micro-organisms in Activated Sludge." *J. Water Polln. Control Fedn., 43,* 604.

Farquhar, G.J. and Boyle, W.C. (1971*b*) "Occurrence of Filamentous Microorganisms in Activated Sludge." *J. Water Polln. Control Fedn., 43,* 779.

Farquhar, G.J. and Boyle, W.C. (1972) "Control of Thiothrix in Activated Sludge." *J. Water Polln. Control Fedn., 44,* 14.

Farrah, S.R. and Unz, R.F. (1976) "Isolation of Exocellular Polymer from Zooglea Strains MP6 and 106 and from Activated Sludge," *Appl. Environ. Microbiol., 32,* 33.

Faust, L. and Wolfe, R.S. (1961) "Enrichment and Cultivation of Beggiatoa Alba." *J. Bacteriol, 81,* 99.

Finstein, M.S. and Heukelekian, H. (1974) "Gross Dimensions of Activated Sludge Flocs with Reference to Bulking." *J. Water Polln. Control Fedn., 39,* 33.

Franson, M., Managing Editor (1985), "Standard Methods for the Examination of Water and Wastewater." American Public Health Association, Washington, DC.

Frenzel, H.J. and Safert, F. (1971) "Erfahren Uber die Verhinderung von Blahschlamm durch Chlorung des belebten Schlammes." *Gas u. Wasserfach, 112,* 604.

Frenzel, H.J. (1977) "Some Experiences with the Chlorination of Activated Sludge for the Fight Against Bulking Sludge in the Berlin-Ruhleben Treatment Plant." *Prog. Water Technol., 8,* 163.

Gaub, Jr., W.H. (1924) "A Bacteriological Study of a Sewage Disposal Plant." *New Jersey Agr. Stat. Bull., No. 394.*

Gaudy, E. and Wolfe, R.S. (1961) "Factors Affecting Filamentous Growth of Sphaerotilus natans." *Appl. Microbiol., 9,* 580.

Gaudy, E. and Wolfe, R.S. (1962) "Composition of an Extracellular Polysaccharide Produced by Sphaerotilus natans." *Appl. Microbiol., 10,* 200.

Goronszy, M.C. (1979) "Intermittent Operation of the Extended Aeration Process for Small Systems." *J. Water Polln. Control Fedn., 51,* 274.

Goronszy, M.C. and Barnes, D. (1979) "Continuous Single Vessel Activated Sludge Treatment of Dairy Wastes." *Proc. 87th Ameri. Inst. Chem. Eng.,* Boston, MA.

Grau, P., Chudoba, J. and Dohanyos, M. (1982) "Theory and Practice of Accumulation-Regeneration Approach to the Control of Activated Sludge Filamentous Bulking." Chapter 7 in *Bulking of Activated Sludge: Preventative and Remedial Methods.* B. Chambers and E.J. Tomlinson, Eds., Ellis Horwood Ltd., Chichester, England.

Greenberg, A.E., Klein, G. and Kaufman, W.J. (1955) "Effect of Phosphorus on the Activated Sludge Process." *Sewage and Ind. Wastes, 27,* 277.

Hale, F.D. and Garver, S.R. (1983) "Viscous Bulking of Activated Sludge." Presented at the 56th Annual Conference Water Polln. Control Fedn., Atlanta, GA, October 2–7.

Hao, O.J. (1980) "Preliminary Report on Nocardia Foaming." Sanitary Engineering and Environmental Health Research Laboratory, University of California, Berkeley.

Hao, O.J. (1982) "Isolation, Characterization and Continuous Culture Kinetics of a New *Sphaerotilus* Species Involved in Low Oxygen Activated Sludge Bulking." Ph.D. Dissertation, Dept. Civil Engineering, University of California, Berkeley, CA.

Hao, O.J., Richard, M.G., Jenkins, D. and Blanch, H. (1983) "The Half Saturation Coefficient for Dissolved Oxygen: A Dynamic Method for its Determination and its Effect on Dual Species Competition." *Biotechnol. Bioengineer, 35,* 403.

Harold, R. and Stanier, R.Y. (1955) "The Genera Leucothrix and Thiothrix." *Bacteriol. Reviews, 19,* 49.

Harris, F.W., Cockburn, T. and Anderson, T. (1927) "Biological and Physical Properties of Activated Sludge." *Waterworks, 66,* 24.

Haseltine, T.R. (1932) "The Activated Sludge Process at Salinas, California, with Particular Reference to Cause and Control of Bulking." *Sewage Works J., 4,* 461.

Hattingh, W.H.J. (1963) "The Nitrogen and Phosphorus Requirements of Micro-organisms." *Water and Waste Treatment, 10,* 380.

Hawkes, H.A. (1960) "Ecology of Activated Sludge and Bacteria Beds." In *Waste Treatment,* edited by P.C.G. Isaac, London: Pergamon Press Ltd.

Heide, B.A., and Pasveer, A. (1974) "Oxidation Ditch: Prevention and Control of Filamentous Sludge." $H_2O$, *7,* 373.

Helmers, E.N., Frame, J.D., Greenberg, A.E. and Sawyer, C.N. (1952) "Nutritional Requirements in the Biological Stabilization of Industrial Wastes." *Sewage and Ind. Wastes, 24,* 496.

Heukelekian, H. and Littman, M.L. (1939) "Carbon and Nitrogen Transformations in the Purification of Sewage by the Activated Sludge Process. I. With Mixtures of Sewage and Activated Sludge." *Sewage Works J., 11,* 226.

_____ II. Morphological and Biochemical Studies of Zooloeal Organisms." *Sewage Works J., 11,* 752.

Heukelekian, H. and Ingols, R.S. (1940) "Studies on Activated Sludge Bulking. II. Bulking Induced by Domestic Sewage." *Sewage Works J., 12,* 694.

Heukelekian, H. (1941) "Activated Sludge Bulking." *Sewage Works J., 19,* 39.

Hohnl, G. (1955) "Investigation into the Physiology and Nutrition of Sphaerotilus natans." *Archiv for Mikrobiologie, 23,* 207.

Houtmeyers, J. (1978) "Relations Between Substrate Feeding Pattern and Development of Filamentous Bacteria in Activated Sludge Processes." *Agricultura* (Belgium), *26,* 1.

Houtmeyers, J., van den Eynde, E., Poffe, R. and Verachtert, H. (1980) "Relations Between Substrate Feeding Pattern and Development of Filamentous Bacteria in Activated Sludge. I. Influence of Process Parameters." *European J. Appl. Microbiol. Biotechnol., 9,* 63.

Hunerburg, K., Sarfert, F. and Frenzel, H.J. (1970) "Ein Beitrag zum Problem Blahschlamm." *Gas. u. Wasserfach., 111,* 7.

Ingols, R.S. and Heukelekian, H. (1940) "Studies on Activated Sludge Bulking. III. Bulking of Sludge Fed with Pure Substances and Supplied with Different Amounts of Oxygen." *Sewage Works J., 12,* 849.

Jenkins, D. (1980) "The Control of Activated Sludge Bulking." Presented at the 52nd Annual California Water Polln. Control Assoc. Conference, Monterey, CA.

Jenkins, D., Parker, D.S., van Neikerk, A.M., Shao, Y-J and Lee, S-E. (1983) "Relationship Between Bench Scale and Prototype Activated Sludge Systems." In *Scale-up of Water and Wastewater Treatment Processes,* ed. Schmidtke, N.W. and Smith, D.W., Butterworth, 307.

Jenkins, D., Richard, M.G. and Neethling, J.B. (1983) "Causes and Control of Activated Sludge Bulking." Presented at the 1983 Annual Conference of the Institute of Water Polln. Control, Southern African Branch, East London, RSA.

Jenkins, D., Richard, M.G. and Daigger, G.T. (1984) "Manual on the Causes and Control of Activated Sludge Bulking and Foaming." Unpublished EPA report. (Subsequent version published in 1986 by the Water Research Commission, Pretoria, Republic of South Africa. Distributed by Ridge Line Press, Lafayette, C.A.).

Johnstone, D.W.M., Rachwal, A.J. and Hanbury, M.J. (1979) 'Settlement Characteristics and Settlement Tank Performance in Carrousel Activated Sludge Systems." *Water Polln. Control, 78,* (3), 337.

Jones, P.H. (1964) "Studies of the Ecology of the Filamentous Sewage Fungus, *Geotrichum candidum.*" Ph.D. Dissertation, Northwestern University, Evanston, IL.

Keefer, C.E. (1963) "Relationship of Sludge Density Index to the Activated Sludge Process." *J. Water Polln. Control Fedn., 35,* 1166.

Keinath, T.M. (1977) "Activated Sludge Unified Design and Operation," *J. Envir. Engr. Div. Amer. Soc. Civil Engng., 103,* 829.

Keller, P.J. and Cole, C.A. (1973) "Hydrogen Peroxide Cures Bulking." *Water and Wastes Engineering, 10,* E4, E6, E7.

Koopman, B. and Cadee, K. (1983) "Prediction of Thickening Capacity Using Diluted Sludge Volume Index." *Water Research, 17,* 1427.

Krul, J.M. (1977) "Experiments with Haliscomenobacter hydrosis in Continuous Culture Without and With Zoogloea ramigera." *Water Research, 11,* 197.

Kraus, L.S. (1945) "The Use of Digested Sludge and Digestor Overflow to Control Activated Sludge Bulking" *Sewage Works J., 170,* 1177.

Lackey, J.B. and Wattie, E. (1946) "The Biology of Sparotilus natans Kutzing in Relation to the Bulking of Activated Sludge." *Sewage Works, 12,* 669.

Larkin, J.M. (1980) "Isolation of Thiothrix in Pure Culture and Observation of a Filamentous Epiphyte on Thiothrix." *Current Microbiol., 4,* 155.

Larkin, J.M. and Strohl, W.R. (1983) "Beggiatoa, Thiothrix and Thioplica." *Ann. Rev. Microbiol., 37,* 341.

Lau, A.O., Strom, P.F. and Jenkins, D. (1984*a*) "Growth Kinetics of spaerotilus natans and a Floc Former in Pure and Dual Continuous Culture." *J. Water Polln. Control Fedn., 56,* 412.

Lau, A.O., Strom, P.F. and Jenkins, D. (1984*b*) "The Competitive Growth of Floc-forming and Filamentous Bacteria: a Model for Activated Sludge Bulking." *J. Water Pollin. Control Fedn., 56,* 52.

Lechevalier, M.P. and Lechevalier, H.A. (1974) "Nocardia amarae sp. nov., an Actinomycete Common in Foaming Activated Sludge." *Inter. J. System Bacteriol., 24,* 278.

Lechevalier, H.A. and Lechevalier, M.P. (1975) *Actinomycetes of Sewage Treatment Plants.* EPA Report No. 600/2-75/031.

Lechevalier, H.A., Lechevalier, M.P. and Wyszkowski, P.E. (1977) *Actinomycetes of Sewage Treatment Plants.* EPA Report 600/2-77-145.

Lee, E.G., Mueller, J.C. and Walden, C.C. (1975) "Effect of Temperature and Sludge Loading on $BOD_5$ Removal and Sludge Settleability in Activated Sludge Systems Treating Bleached Kraft Effluents." *TAPPI, 58,* 100.

Lee, S-E., Koopman, B.L., Jenkins, D. and Lewis, R.F. (1982) "The Effect of Aeration Basin Configuration on Activated Sludge Bulking at Low Organic Loading." *Water Sci. and Technol., 14,* 407.

Lee, S-E., Koopman, B.L., Bode, H., and Jenkins, D. (1983) "Evaluation of Alternative Sludge Settleability Indices." *Water Research, 17,* 1421.

Lemmer, H. and Kroppenstedt, R.M. (1984) "Chemotaxonomy and Physiology of some Actinomycetes Isolated from Scumming Activated Sludge." *System. Appl. Microbiol., 5,* 124.

Lewis, R.F. (1979) Privately communicated. U.S.E.P.A., A.W. Briedenbach Environmental Research Center, Cincinnati, Ohio.

Matsche, N.F. (1977) "Blahschlamm-Versuchen and Bekaufung-Weiner Mittelunger." *Wasser-Abwasser-Gewasser, 22,* 1.

Matsche, N.F. (1982) "Control of Bulking Sludge—Practical Experience in Austria." *Water Sci. and Technol., 14,* 311.

Matsche, N. (1980) Privately communicated Dept. of Sanitary Engineering, Technishce Universiteit of Vienna, Austria.

Matsui, S. and Yamamoto, R. (1983) "Measuring the Lengths of Filamentous Microbes in Activated Sludges using a Video Color T.V. with a Microscope." Presented at International Assoc. Water Polln. Research and Control Workshop on Design and Operation of Large Wastewater Treatment Plants. Sept. 19–23, 1983, Vienna, Austria.

McKinney, R.E. and Horwood, M.P. (1952) "Fundamental Approach to the Activated Sludge Process. I. Floc-Producing Bacteria." *Sewage Ind. Wastes, 24,* 117.

McKinney, R.E. and Weichlein, R.G. (1953) "Isolation of Floc-Producing Bacteria from Activated Sludge." *Appl. Microbiol., 1,* 259.

McKinney, R.E. (1955) *Bacterial Flocculation in Relationship to Aerobic Waste Treatment Processes.* Nat. Inst. of Health, Progress Rpt. Proj. E. 645 Mass. Inst. Technol., Cambridge, MA.

McKinney, R.E. (1957) "Activity of Micro-organisms in Organic Waste Disposal. II. Aerobic Processes." *Appl. Microbiol., 5,* 167.

Merkel, G.J. (1975) "Observations on the Attachment of Thiothrix to Biological Surfaces in Activated Sludge." *Water Research, 9,* 881.

Miyamoto-Mills, J., Larson, J., Jenkins, D., and Owen, W.F. (1983) "Design and Operation of a Pilot-Scale Biological Phosphate Removal Plant at Central Contra Costa Sanitary District, CA." *Water Sci. and Technol.* (Capetown), *15,* 153.

Mohlman, F.W. (1934) "The Sludge Index." *Sewage Works J., 6,* 119.

Mulder, E.G. (1964) "Iron Bacteria, Particularly Those of the Spaerotilus-Leptothrix Group, and Industrial Problems." *J. Appl. Bacteriol., 27,* 151.

Mulder, E.G. and Van Veen, W.L. (1963) "Investigations on the Sphaerotilus-Leptothrix Group." *Antonie van Leeuwenhoek, 29,* 121.

Neethling, J.B., Johnson, K.M. and Jenkins, D. (1982) *Chemical and Microbiological Aspects of Filamentous Bulking Control Using Chlorination.* UCB-SEEHRL Report No. 82-2, Sanitary Engineering and Environmental Health Research Laboratory, College of Engineering and School of Public Health, University of California, Berkeley, CA.

Neethling, J.B. (1984) "The Control of Activated Sludge Bulking by Chlorination." Ph.D. Dissertation, Dept. of Civil Engng., Univ. of Calif. Berkeley, CA.

Neilsen, P.H. (1984) "Oxidation of Sulfide and Thiosulfate and Storage of Sulfur-Granules in Thiothrix from Activated Sludge." Presented at the 12th Biennal Conference Intl. Assoc. for Water Polln. Res., Amsterdam, Holland.

Nurdogan, Y. and Jenkins, D. (1983) Unpublished data Sanitary Engineering and Environmental Health Research Laboratory, University of Calif., Berkeley, CA.

Palm, J.C., Jenkins, D. and Parker, D.S. (1980) "Relationship Between Organic Loading, Dissolved Oxygen Concentration and Sludge Settleability in the Complete-Mixed Activated Sludge Process." *J. Water Polln. Control Fedn., 52,* 2484.

Parker, D.S., Jenkins, D. and Kaufman, W.J. (1971) "Physical Conditioning of the Activated Sludge Floc." *J. Water Polln. Control Fedn., 43,* 1817.

Parker, D.S., Jenkins, D. and Kaufman, W.J. (1972) "Floc Breakup in Turbulent Flocculation Processes." *J. Sanitary Eng. Div., Amer. Soc. Civil Engng., 98,* SAI, 79.

Pavoni, J.L., Tenney, M.W. and Echelberger, W.T. Jr. (1972) "Bacterial Exocellular Polymers and Biological Flocculation," *J. Water Polln. Control Fedn., 44,* 414.

Pearse, L. and APHA Committe (1937) "Bulking of Sludge in the Activated Sludge Process of Sewage Treatment." *APHA Year Book,* (Supplement to the Journal), *27,* 164.

Peptiprez, M. and Leclerc, H. (1969) "Les Baeries du Group Sphaerotilus-Leptothrix." *Ann. Inst. Pateur Lille, 20,* 115.

Phaup, J.D. (1968) "The Biology of Sphaerotilus Species." *Water Research, 2,* 597.

Pike, E.G. (1972) "Aerobic Bacteria" in *Ecological Aspects of Used Water Treatment, 1,* C.R. Curds and H.A. Hawkes (Eds), Academic Press, N.Y. pp. 1–63.

Pipes, W.O. and Jones, P.H. (1963) "Decomposition of Organic Wastes by Sphaerotilus." *Biotech. and Bioeng., 5,* 287.

Pipes, W.O. (1974) "Control Bulking with Chemicals." *Water and Wastes Engineering, 9,* 30.

Pipes, W.O. (1978) "Actinomycete Scum Formation in Activated Sludge Processes." *J. Water Polln. Control Fedn,, 5,* 628.

Pipes, W.O. (1979) "Bulking, Deflocculation and Pin-Point Floc." *J. Water Polln. Control Fedn., 51,* 62.

Pittman, A.R. (1984) "Settling of Nutrient Removal Activated Sludges." Presented at 12th Biennal Conference Intl. Assoc. for Water Polln. Res., Amsterdam, Holland.

Pringsheim, E.G. (1949) "The Filamentous Bacteria Sphaerotilus, Leptothrix, Cladothrix and their Relation to Iron and Manganese." *Phil. Trans. Roy. London, 233B,* 453.

Pringsheim, E.G. (1951) "The Vitreoscilla: A Family of Colorless, Gliding Filamentous Organisms." *J. Gen. Microbiol., 5,* 124.

Pringsheim, E.G. (1964) "Heterotrophism and Species Concept in Beggiatoa." *Amer. J. Botany, 51,* 898.

Pringsheim, E.G. and Wiessner, W. (1963) "Minimum Requirements for Heterotrophic Growth and Reserve Substances in Beggiatoa." *Nature (London), 197,* 102.

Poffe, R., van der Leyden, J. and Verachtert, H. (1979) "Characterization of a Leucothrix-Like Bacterium Causing Sludge Bulking During Petro-Chemical Waste Water Treatment." *European J. Microbiol. Biotechnol., 8,* 229.

Rachwal, A.J., Johnstone, D.W.M., Hanbury, M.J., Critchard, D.J. (1982) "The Application of Settleability Tests for the Control of Activated Sludge Plants." Chapter 13 in *Bulking of Activated Sludge: Preventative and Remedial Methods,* B. Chambers and E.J. Tomlinson, Eds. Ellis Horwood Ltd., Chichester, England.

Rensink, J.H. (1974) "New Approach to Preventing Bulking Sludge." *J. Water Polln. Control Fedn., 46,* 1888.

Rensink, J.H., Leentvaar, J. and Donker, H.J.G.W. (1979) "Control of Bulking Combined with Phosphate Removal by Ferrous Sulphate Dosing." $H_2O$, *12,* 150.

Rensink, J.H., Donker, H.J.G.W., and Ijwema, T.S.J. (1982) "The Influence of Feed Pattern on Sludge Bulking." Chapter 9 in *Bulking of Activated Sludge: Preventative and Remedial Methods,* B. Chambers and E.J. Tomlinson, Eds. Ellis Horwood Ltd., Chichester, England.

Richard, M.G., Jenkins, D., Hao, O. and Shimizu, G. (1982*a*) *The Isolation and Characterization of Filamentous Micro-organisms from Activated Sludge Bulking.* Report No. 81-2, Sanitary Engineering and Environmental Health Research Laboratory, Univ. of Calif., Berkeley, CA.

Richard, M.G., Hao, O. and Jenkins, D. (1982*b*) "Growth Kinetics of Sphaerotilus Species and their Significance in Activated Sludge Bulking." Presented at the 55th Annual Conference of the Water Polln. Control Fedn., St. Louis, MO.

Richard, M.G., Shimizu, G., Jenkins, D., Williams, T. and Unz, R.F. (1983) "Isolation and Characterization of Thiothrix and Thiothrix-Like Filamentous Organisms from Bulking Activated Sludge." Presented at the 1983 Annual Meeting of the Amer. Soc. for Microbiology, New Orleans, LA (*Abs. Ann. Meet., 060,* 270).

Richard, M.G., Shimizu, G. and Jenkins, D. (1984) "The Growth Physiology of the Filamentous Organism type 021N and its Significance to Activated Sludge Bulking." Presented at the 57th Annual Conference, Water Polln. Control Fedn., New Orleans, LA.

Richard, M.G. (1984) "Filamentous Organism Identification Training Sessions," Report to the Water Research Commission, Republic of South Africa.

Rouf, M.A. and Stokes, J.L. (1964) "Morphology, Nutrition and Physiology of Sphaerotilus discophorus." *Arch. fur Mikrobiologie, 49,* 132.

Ruchhoft, C.C. and Kachmar, J.F. (1941) "Studies of Sewage Purification. XIV. The Role of Sphaerotilus natans in Activated Sludge Bulking." *Sewage Works J., 13,* 3.

Scotten, H.L. and Stokes, J.L. (1962) "Isolation and Properties of Beggiatoa." *Archiv fur Mikrobiologie, 42,* 353.

Sezgin, M. (1977) "The Effect of Dissolved Oxygen Concentration on Activated Sludge Process Performance." Ph.D. Thesis, Univ. of Calif., Berkeley, CA.

Sezgin, M., Jenkins, D. and Parker, D.S. (1978) "A Unified Theory of Filamentous Activated Sludge Bulking." *J. Water Polln. Control Fedn., 50,* 362.

Sezgin, M. (1980) "The Role of Filamentous Microorganisms in Activated Sludge Settling." *Prog. Water Technol., 12,* 97.

Sladka, A. and Ottova, V. (1973) "Filamentous Organisms in Activated Sludge." *Hydrobiologia, 43,* 285.

Slijkhuis, H. and Deinema, M.H. (1982) "The Physiology of Microthrix parvicella, a Filamentous Bacterium Isolated from Activated Sludge." Chapter 5 in *Bulking of Activated Sludge: Preventative and Remedial Methods.* B. Chambers and E.J. Tomlinson, Eds., Ellis Horwood Ltd., Chichester, England.

Slijkhuis, H. (1983) "The Physiology of the Filamentous Bacterium Microthrix parvicella." Ph.D. thesis, Wageningen, Holland.

Slijkhuis, H. (1983) "Microthrix parvicella, a Filamentous Bacterium Isolated from Activated Sludge: Cultivation in a Chemically Defined Medium." *Appl. Environ. Microbiol., 46,* 832.

Smit, J. (1932) "A study of the Conditions Favoring Bulking of Activated Sludge." *Sewage Works J., 4,* 960.

Smit, J. (1934) "Bulking of Activated Sludge. II. Causative Organisms." *Sewage Works J., 6,* 1041.

Smith, R.S. and Purdy, W.C. (1936) "The Use of Chlorine for the Correction of Sludge Bulking in the Activated Sludge Process." *Public Health Reports, 51,* 20, 617.

Spector, M.L. (1977) "Production of Non-Bulking Activated Sludge." U.S. Patent 4,056,465.

Steytler, R.B., Goddard, M.F., Ambrose, W.A. and Wilson, T.E. (1981) "Listening to the Activated Sludge Plant in Phoenix." Presented at the 54th Annual Conference, Water Polln. Control Fedn., Detroit, MI.

Steytler, R., Goddard, M.F., Buhr, H.O. and Wilson, T.E. (1982) "Watching the 91st Avenue Plant in Phoenix." Presented at the 55th Annual Meeting, Arizona Water Polln. Control Assoc., Flagstaff, AZ.

Stobbe, G. (1964) "Uber das Verhalten von belebtem Schlamn in aufsteigender Wasserbewegung." Berofrentlichungen, des Institutes fur Siedlungswasserwirtshaft der Technischen Hochschule Hannover, Heft 18.

Stokes, J.L. (1984) "Studies on the Filamentous Sheathed Bacterium Sphaerotilus natans." *J. Bacteriol, 67,* 278.

Strohl, W.R. and Larkin, J.M. (1978) "Enumeration, Isolation and Characterization of Beggiatoa from Freshwater Sediments." *Appl. Environ. Microbiol., 36,* 755.

Strom, P.F. and Finstein, M.S. (1977) "Nitrification in a Chlorinated Activated Sludge Culture." *J. Water Polln. Control Fedn., 49,* 584.

Strom, P.F. and Jenkins, D. (1984) "Identification and Significance of Filamentous Micro-organisms in Activated Sludge." *J. Water Polln. Control Fedn., 56,* 52.

Strunk, W.G. and Shapiro, J. (1976) "Bulking Control Made Easy—With Hydrogen Peroxide." *Water Polln. Control, 11.*

Tabor, W.A. (1976) "Wastewater Microbiology." *Ann. Rev. Microbiol, 30,* 263.

Tago, Y. and Aiba, K. (1977) "Exocellular Mucopolysaccharide Closely Related to Bacterial Floc Formation." *Appl. Environ. Microbiol. 34,* 308.

Tapleshay, J.A. (1945) "Control of Sludge Index by Chlorination of Return Sludge." *Sewage Works J., 17,* 1210.

Thirion, N. (1983) "Chlorination as a Control Measure for Bulking Sludge at the Dasport Sewage Treatment Plant, Pretoria." *SA Water Bulletin,* Water Research Commission, Pretoria, RSA.

Tomlinson, E.J. (1976) *Bulking: A Survey of Activated Sludge Plants.* Technical Report TR35, Water Research Centre, Stevenage, England.

Tomlinson, E.J. (1978) *The Effect of Anoxic Mixing Zones upon the Settleability of Activated Sludge.* Unpublished Report. Water Research Centre, Stevenage, England.

Tomlison, E.J. and Chambers, B. (1978*a*) *The Use of Anoxic Mixing Zones to Control the Settleability of Activated Sludge.* Technical Report TR116, Water Research Centre, Stevenage, England.

Tomlinson, E.J. and Chambers, B. (1979) "Methods for Prevention of Bulking in Activated Sludge." *Water Polln. Control, 78,* 524.

Tomlinson, E.J. (1982) "The Emergence of the Bulking Problem and the Current Situation in the U.K." Chapter 1 in *Bulking of Activated Sludge: Preventative and Remedial Methods,* B. Chambers and E.J. Tomlinson, Eds., Ellis Horwood Ltd., Chichester, England.

Torpey, W.N. (1948) "Practical Results of Step Aeration." *Sewage Works J., 20,* 781.

Torpey, W.N. and Chasick, A.H. (1955) "Principles of Activated Sludge Operation." *Sewage Works J., 27,* 1217.

Tucek, F. and Chudoba, J. (1969) "Purification Efficiency in Aeration Tanks with Complete Mixing and Piston Flow." *Water Research, 3,* 559.

Unz, R. (1979) Privately communicated. Dept. Civil Engineering, The Pennsylvania State University, State College, PA.

Unz, R.F. and Farah, S.R. (1976) "Exopolymer Production and Flocculation by Zoogloea MP6." *Appl. Environ. Microbiol., 31,* 623.

van den Eynde, E., Vriens, L. and Verachtert, H. (1982*a*) "Relation Between Substrate Feeding Pattern and Development of Filamentous Bacteria in Activated Sludge Processes. III. Application with Industrial Waste Waters." *European J. Appl. Microbiol. Engineer. 15,* 246.

van den Eynde, E., Houtmeyers, J. and Verachtert, H. (1982*b*) "Relation Between Substrate Feeding Pattern and Development of Filamentous Bacteria in Activated Sludge." In *Bulking of Activated Sludge: Preventative and Remedial Methods,* B. Chambers and E.J. Tomlinson, Eds., Ellis Horwood Ltd., Chichester, England.

van den Eynde, E., Geerts, J., Maes B. and Verachtert, H. (1983) "Influence of the Feeding Pattern on the Glucose Metabolism of Arthrobacter spp. and Sphaerotilus natans, Growing in Chemostate Culture, Simulating Activated Bulking." *European J. Appl. Microbiol. and Biotechnol., 17,* 35.

van den Eynde, E. (1983) "Substrate Stress: A Condition for Safe Operation of Activated Sludge Systems with Regard to Filamentous Bulking." Ph.D. Thesis, Katholieke Universiteit de Leuven, Belgium.

van Neikerk, A.M., Wright, A., Kawahigashi, J. and Reichlin, D. (1982) *Digestor Foaming Problems and their Causes at the East Bay Municipal Utility District Plant, Oakland, California.* Class Report to Professor D. Jenkins, CE 298-5, Univ. of Calif., Berkeley, CA.

van Niekerk, A.M., Jenkins, D. and Richard, M.G. (1984) Unpublished data. Sanitary Engineering and Environmental Health Research Laboratory, Univ. of Calif., Berkeley, CA.

van Veen, W.L. (1972) "Factors Affecting the Oxidation of Manganese by Sphaerotilus discophorus." *Antonie van Leeuwenhoek, 38,* 623.

van Veen, W.L., van der Kooij, J., Gruze, E.C.W.A. and van der Vlies, A.W. (1973) "Investigations of the Sheathed Bacterium Haliscomenobacter hydrosis, a Bacterium Occurring in Bulking Activated Sludge." *Antonie* van Leeuwenhoek, 39, 207.

van Veen, W.L., Krul, J.M. and Bulder, C.J.E.A. (1982) "Some Growth Parameters of Haliscomenobacter hydrosis, a Bacterium Occurring in Bulking Activated Sludge." *Water Research, 16,* 531.

Vega-Rodriquez, B.A. (1983) "Quantitative Evaluation of Nocardia spp. Presence in Activated Sludge." M.S. Thesis, Civil Engineering, Univ. of Calif., Berkeley, CA.

Verachtert, H., van den Synde, E., Poffe, R. and Houtmeyers, J. (1980) "Relations Between Substrate Feeding Pattern and Development of Filamentous Bacteria in Activated Sludge Processes. II. Influence of Substrate Present in the Influent." *European J. Appl. Microbiol. Biotechnol., 9,* 137.

Wagner, F. (1982) "Study of the Causes and Prevention of Sludge Bulking in Germany." Chapter 2 in *Bulking of Activated Sludge: Preventative and Remedial Methods,* B. Chambers and E.J. Tomlinson, Eds., Ellis Horwood Ltd., Chichester, England.

Waitz, S. and Lackey, J.B. (1959) "Morphological and Biochemical Studies on the Organisms Sphaerotilus natans." *Otrly. J. Fla. Acad. of Sci., 21,* 335.

Weddle, C.L. and Jenkins, D. (1971) "The Viability and Activity of Activated Sludge." *Water Research, 5,* 621.

Weiches, H.N.S. (1983) "Sludge Bulking in Heidelberg." SA Water Bulletin, Water Research Commission, Pretoria, RSA.

Wells, W.N. and Garret, M.T. Jr. (1971) "Getting the Most from an Activated Sludge Plant." *Pub. Works, 63.*

Wheeler, D.R. and Rule, A.M. (1980) "The Role of Nocardia in the Foaming of Activated Sludge: Laboratory Studies." Presented at Georgia Water and Polln Control Assoc. Annual Meeting, Savannah, GA.

Wheeler, M., Jenkins, D. and Richard, M.G. (1983) "The Use of Selectors for Bulking Control at the Hamilton, Ohio, USA, Water Polln. Control Facility." Proceedings, Workshop on Design and Operation of Large Wastewater Treatment Plants, Intl. Assoc. for Water Polln. Res. and Control, Vienna, Austria.

White, M.J.D. (1975) *Settling of Activated-Sludge.* Technical Report TR11, Water England.

White, M.J.D. (1976) "Design and Control of Secondary Settlement Tanks." *Water Polln. Control, 75,* 459.

White, M.J.D., Tomlinson, E.J. and Chambers, B. (1980) "The Effect of Plant Configuration on Sludge Bulking." *Progress in Water Technol,* 183.

Williams, T.M. and Unz, R.F. (1983) "Environmental Distribution of Zoogloean Strains." *Water Research, 17,* 779.

Wilson, T.E., Ambrose, W.A., and Buhr, H.O. (1983) "Operating Experiences SRT" Proceedings Workshop on Design and Operation of Large Wastewater Treatment Plants, *Intl Assoc. for Water Polln. Res. and Control,* Austria.

Wilson, T.E. (1983) "Application of the ISV Test to the Operation of Sludge Plants." *Water Research, 17,* 707.

Wood, D.K. and Tchobanoglous, G.T. (1974) "Trace Elements in Biological Treatment with Specific Reference to the Activated Sludge Process." Presented at the 29th Industrial Waste Conference, Purdue University, IN.



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